Studies to develop a laparoscopic instrument for obtaining diagnostic quality full thickness intestinal biopsies in horses

by

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Abstract

Currently, there is no widely used minimally invasive technique in human or veterinary medicine, which allows for intracorporeal full thickness intestinal biopsy (FTIB). The aim of this study was to investigate the design of a prospective laparoscopic FTIB instrument including an exploration of techniques to excise and close the biopsy excision site (BES). This was achieved by a preliminary investigation. This involved the identification of the possible techniques; screening of the techniques based on a scoring system devised from the desired requirements of the instrument; and then a short list of the techniques were evaluated by an appropriate test. This included a histological examination to determine the optimal technique for excision and intra-luminal bursting pressures to assess the BES closure method. The optimal biopsy excision technique (BET) was then adopted as part of a prototype biopsy instrument and used to obtain 30 biopsies from the distal jejunum and ileum of three horses, which had been euthanized for reasons unrelated to gastrointestinal disease. The histological quality of the biopsy sample was assessed by a boarded histopathologist and scored 1 (Excellent) - 6 (Very Poor). The BES closure method was incorporated as part of a prototype instrument and evaluated by intra-luminal bursting pressures. The positive attributes and potential modifications of the instrument are reported. The optimal excision shape and technique was a U-shape used in a “chopping board-like” technique (CBT). The prototype instrument obtained an average biopsy score of 3.46 (Five biopsy scored very good, twelve biopsy scored good, six scored acceptable and seven scored poor). A lack of mucosa or disruption of the mucosal layer was the most common reason for down grading of samples. Myenteric ganglia and/or submucosal ganglia were present in all of the Prototype 2 biopsy samples. The U-shaped BET used as part of the CBT excision technique has the potential to be used as part of a laparoscopic instrument to obtain full thickness intestinal biopsy from the intestine and a curved double row of staples used in a manner similar to a linear stapler offer a potential closure method as part of a one step laparoscopic instrument.

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Author's Declaration

I, Bryan J. O'Meara, declare that the work in this thesis is original, was carried out solely by myself or with due acknowledgments. It has not been submitted in any form for another degree or professional qualification.

Bryan J. O'Meara

Part of this thesis was presented elsewhere:

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List of Abbreviations and Definitions

BES Biopsy Excision Site – the site at which the intestinal biopsy was excised from

BET Biopsy Excision Technique – the technique used to excise the intestinal biopsy

BWT Bowel Wall Thickness – the distance between the calipers when placed with light finger pressure on the folded longitudinal border located midway between the anti-mesenteric and mesenteric border of the intestine; the arms of the calipers must extend as far as the mid lumen of the intestine.

CBT "Chopping Board-Like" Technique – a technique in which the anti-mesenteric edge of a folded section of intestine was presented so that the two full thickness layers of the intestine were excised in one folded sample in a chopping-like action or by similar means against a hard surface

FTIB Full thickness intestinal biopsy – a sample containing all the layers of intestine i.e. mucosa, submucosa, muscularis and serosal layers

GE Granulomatous Enteritis

EC Idiopathic Eosinophilic Enterocolitis

IBD Inflammatory Bowel Disease

HD Hirschsprung Disease

LPE Lymphocytic/Plasmacytic Enterocolitis

MEED Multisystemic Eosinophilic Epitheliotrophic Disease

Chapter 1. Introduction

Diagnosis of intestinal disease in the horse is a rather complex problem due to its anatomic location within the abdomen enveloped by 18 pairs of ribs and the vast size of the intestine (i.e. the small intestine measures between 10 and 30 metres in a horse (White *et al*. 2008)). Diagnostic imaging of the horse's abdomen is compromised by the size of the horse in relation to the equipment available i.e. no computed tomography or magnetic resonance imaging unit are large enough for abdominal examinations in adult horses. Furthermore radiography although sometimes useful is generally not performed due to the large body mass and the fact that radiation scatter makes the quality of the image difficult to interpret (Yarbrough *et al*. 1994; Vatistas *et al*. 1996; Ruohoniemi *et al*. 2001; Granot *et al*. 2008; Kendall *et al*. 2008; Rowe and White 2008). Transabdominal ultrasonography and gastroscopy are employed routinely in equine veterinary practice (Anthony *et al*. 2004, Rowe and White 2008). Other methods to investigate intestinal disease, which do not involve histological assessment, include abdominal auscultation, physical examination, clinical pathology, trans-rectal palpation, faecal examination, nuclear scintigraphy and although infrequently performed, tests of absorption and digestion and evaluations of gastric emptying by radiography or scintigraphy (Freeman 2006a). It should be noted, however, that although the information gleaned from these modalities give valuable information on intestinal pathology, they fail to assess in full measure the structure of the organ and its histomorphology and secretory function (Avunduk 2008).

Biopsy of the intestine allows for a histological diagnosis. The portion of the intestine sampled and the layers sampled determine the usefulness of the biopsy as regards diagnosis. A partial thickness biopsy of the duodenum obtained by a 3-metre endoscope or that obtained from the rectum by a rectal biopsy forceps allows for examination of the inner layers (mucosa and submucosa primarily) of the equine intestine. The specificity of these tests must be questioned in light of the length of the equine small intestine being an average of 25 metres (Freeman 2006) and the sample may only be representative of the sampled portion (Mair *et al*. 2006). For example rectal biopsy are unlikely to be diagnostic in eosinophilic enterocolitis (EC) and lymphocytic plasmacytic enteritis (LPE) and has been shown to have a sensitivity of 50% in cases of granulomatous enteritis (GE) (9/18) and

multicentric eosinophilic epitheliotrophic disease (MEED) (6/12) (Lindberg 1986; Schumacher *et al*. 2000a; Packer *et al*. 2005). Furthermore it is logical to conclude that partial thickness biopsy would be successful in the diagnosis of alimentary neoplasia involving the mucosa and submucosa (i.e. lymphoma and adenocarcinoma) however partial thickness intestinal biopsy is not useful in the diagnosis of Equine Dysautonomia (Wood *et al*. 1998; Doxey *et al*. 1999; Hahn and Mayhew 2000). Often if a diagnosis cannot be established by the aforementioned methods an exploratory laparotomy or laparoscopy is carried out to investigate abdominal disease in the horse and to allow for excision of a full thickness intestinal biopsy (FTIB) (Hendrickson 2000). FTIB of the horse is indicated to evaluate such conditions as inflammatory bowel disease (IBD), neoplasia and Grass Sickness (Freeman 2006). The most common indication for FTIB at the Weipers Centre Equine Hospital, University of Glasgow is in the diagnosis of Grass Sickness (Equine Dysautonomia). The disease is most prevalent in Scotland and north of England (Proudman 2005). The histological areas of interest are the submucosal and myenteric ganglia. Endoscopically obtained and suction biopsies as obtained in human medicine are partial thickness and do not allow sufficient examination of the ganglia (Avunduk 2008).

A FTIB encompasses all of the intestinal layers and allows for an optimal histological examination; this is most frequently achieved in the horse by exteriorisation of the intestine and excision of the biopsy. This procedure is most usually performed whilst the horse is anaesthetised via a ventral midline abdominal celiotomy incision as part of an exploratory laparotomy (Fischer 2002). There is a high risk of mortality with general anaesthesia in the horse (Johnston 2005). More recently a laparoscopic aided procedure results in identification of small intestine and exteriorisation of small intestine via a flank laparotomy with subsequent intestinal biopsy excision similar to conventional surgical technique (Fischer 2002). Other techniques in human medicine and more recently in horses have involved the use of a laparoscopic linear stapler to excise a fold of intestine (Evans *et al*. 2006). These techniques are not widely carried out; perhaps this is due to the perception that a large section of intestine is being excised and thus the healing biopsy site has the potential to cause a luminal stenosis (Freeman 2006) furthermore it is conceivable that the placement of a linear stapler is

perceived to be difficult due to the two dimensional information on a video screen.

Aim

The purpose of the study was to design a minimally invasive laparoscopic instrument and technique for full thickness intestinal biopsy (FTIB) in the horse.

"After identifying a limitation in the minimally invasive procedure, a technical problem specification is formulated to serve as the starting point for the design."

The above quotation is from an article entitled "The Development of Laparoscopic Instruments" in the journal Minimally Invasive Therapy and Allied Technologies by Grimbergen 2001. The project was initiated by the lack of a reliable and widely used laparoscopic one step intracorporeal FTIB instrument for use in the horse. The limitations in the design of a laparoscopic FTIB instrument are the methods by which the excision of the biopsy and closure of the excision site is carried out. The biopsy should be small in size yet offer an excellent quality biopsy for histological examination.

Preliminary investigations were carried out to develop a biopsy excision technique (BET) and a biopsy excision site (BES) closure technique (Appendices 3 and 4). These techniques were incorporated as part of prototype instrument.

The first hypothesis was that the new biopsy excision technique incorporated as part of a prototype instrument was capable of achieving biopsy quality comparable to controls. The second hypothesis was that the chosen BES closure technique incorporated as part of a prototype instrument was capable of achieving closure of the BES comparable to a gold standard technique.

Chapter 2. Literature Review

Equine laparoscopy

Surgical advances have created new techniques and surgical equipment that can overcome the morbidity associated with large incisions (Hendrickson 2000; Nagle *et al*. 2004; Mayhew 2009). Over the last 15 years, laparoscopic or laparoscopic hand-assisted procedures have been used with increasing frequency both in human and equine patients (Fischer 2002). A solely laparoscopic surgery is where abdominal surgery is performed using only small incisions, about 5–20mm in diameter and the procedure is carried out completely within the abdominal cavity using a laparoscopic camera for visualisation and long handled instruments for manipulation. In contrast to ‘‘open’’ surgery where surgeons manually manipulate and palpate (touch) the tissue or organ during the operation, any procedure performed laparoscopically is carried out using a laparoscopic instrument designed to fit into the body through guides called cannula (Galuppo *et al*. 1995). Walmsley (1999) and Galuppo *et al.*, (1995) described the laparoscopic anatomy of the standing horse via flank portals. Laparoscopy can be a useful diagnostic tool in horses with acute and chronic colic. In horses with acute colic, laparoscopy can confirm the need for exploratory laparotomy or euthanasia. A sensitivity of 82% was found for horses with acute colic, coupled with a specificity of 66% indicating that if a lesion was found during laparoscopy it was the likely cause, however a significant number of lesions were missed (Fischer 2002). The sensitivity for lesion identification in horses with chronic colic declined to 63% with a specificity of 17% (Galuppo *et al*. 1996; Rawlings *et al*. 2002). Although the laparoscopic examination of the abdomen in dorsal recumbency was described in horses and also small animals; this surgery involves placement of a paramedian portal lateral to the right rectus abdominis muscle and it is infrequently carried out (Freeman 2006). Acute colic lesions are rarely treated solely by laparoscopy and perhaps this is one of the reasons that laparoscopy is not frequently carried out in favour of exploratory laparotomy whilst the horse is anaesthetised and in dorsal recumbency (Fischer 2002). There are many laparoscopic procedures now routinely carried out in the standing horse, for example laparoscopic cryptorchid castration, ovariectomy, exploratory laparoscopy and closure of the nephrosplenic space (Hendrickson 2000; Fischer 2002; Smith *et al*. 2005). Hand-assisted laparoscopic surgeries

involve the use of a laparoscope to visualize the surgery site for example, instruments are used to manipulate a tissue and a hand is used to retrieve or aid in the procedure via a flank incision (Fischer 2002).

Most recently there have been many new intracorporeal techniques described including single-site laparoscopic surgery, needlescopic surgery, robotic surgery, natural orifice transluminal endoscopic surgery (NOTES) and alimentary endoscopically assisted laparoscopy (Halim and Tavakkolizadeh 2008; Ponsky and Ponsky 2009). NOTES procedures via the vulva, anus or mouth or sometimes combined with an incision through the vagina, rectum or stomach or as a laparoscopic assisted surgery via the umbilicus (Ponsky and Ponsky 2009). In recent years NOTES procedures in horses have been cited in the literature (Alford and Hanson 2010, Pader *et al*. 2011a, Pader *et al*. 2011b*)*. To fulfil the need for surgical instruments to allow advances in minimally invasive procedures, there is a need for a reliable design methodology and sets of analytical tools for designing new or redesigning existing instruments (Grimbergen 1997). Laparoscopic procedures remain highly technical, requiring specialized and expensive instrumentation as well as considerable training to decrease the risks associated with the procedure (Schambourg and Marcoux 2006).

Currently, the most widely used techniques to obtain FTIB in the horse are by ventral midline laparotomy or as a standing laparoscopic assisted surgery via a flank incision (Fischer 2002). A ventral midline laparotomy traditionally achieved by a 30cm or greater in length incision allows for exteriorisation of 75% of the intestinal tract and subsequent FTIB excision using conventional surgical techniques (White *et al*. 2008). Alternative approaches to the abdomen include ventral paramedian, inguinal and flank approach (paralumbar incision, transverse flank incision) (Fischer 2002). A standing laparoscopic assisted surgery to obtain a FTIB of the small intestine requires laparoscopic portals to locate the intestine and subsequently a 10cm flank incision is needed to allow exteriorisation and the FTIB is obtained in a conventional manner (Schambourg and Marcoux 2006). Flank incisions have a greater rate of surgical site infection than ventral midline celiotomy (88% versus 29%) (Wilson 1995). A ventral median celiotomy requires 8 weeks to heal and re-laparotomy is a recognized risk factor for infection and hernia formation (French *et al*. 2002). Sequential intestinal

biopsies may also be required for experimental studies and a less invasive approach would decrease cost and the risk of complications especially if biopsies were collected sequentially, a few weeks apart (Schambourg and Marcoux 2006). In the study by Schambourg and Marcoux (2006) postoperative care was minimal, and surgical cost was approximately one-third the cost of elective ventral midline celiotomy in the four horses from which FTIB were obtained. There is no risk of herniation or evisceration with a solely laparoscopic technique. This should allow for immediate use of corticosteroid therapy if required to treat the primary condition, as is usually indicated in chronic IBDs. This treatment has to be postponed for several weeks after a ventral midline laparotomy (Fischer 2002). A standing laparoscopic surgery negates the risk of general anaesthesia in horses of which the non-colic associated mortality was reported to be 0.9% in a large-scale, multi-centre study (Johnston and Steffey 1995) while the perianaesthetic mortality rate at a single, busy equine surgical practice was somewhat more favourable, at 0.12% (Bidwell *et al*. 2007). Furthermore the catabolic state of many horses (for example Grass Sickness) that may require intestinal biopsy make them potentially poor candidates for general anaesthesia and normal healing after ventral midline celiotomy. The benefit of a definitive diagnosis by a FTIB may limit the unnecessary suffering for an individual in which a diagnosis is reached in a shorter time (Proudman 2005).

The technique to examine the small intestine described by Schambourg and Marcoux (2006) used one laparoscope portal and two instrument portals which were located in the right flank: instrument portal one was positioned half-way between the tuber coxae and the last rib at the level of the crus of the internal abdominal oblique muscle, the laparoscope portal was positioned at the level of the 17th intercostal space, 20cm distal to the transverse process of the lumbar vertebrae, and instrument portal two was positioned 25–30cm ventral to portal one in the flank. Schambourg and Marcoux (2006) required a third instrument portal, which was positioned 25–30cm ventral to the laparoscope portal, in the 16th intercostal space; this portal was used to introduce a laparoscopic scissors and a needle-holder. Thus there were four laparoscopic portals were required in the right flank in the Schambourg and Marcoux (2006) technique. During laparotomy the jejunum can be exteriorized entirely, but the duodenum and the distal part of the ileum can not be retracted to the laparotomy incision to be

biopsied (Fischer 2002; Schambourg and Marcoux 2006). It was determined that a laparoscopic intestinal exploration from the right flank allowed manipulation of 40cm more ileum compared with ventral midline celiotomy (Schambourg and Marcoux 2006). Only the jejunum and the proximal ileum can be exteriorized through a flank incision in horses, therefore this technique is not applicable to lesions localized to either the duodenum or most of the ileum (Bracamonte *et al*. 2008).

Histopathological areas of interest in the horse

Biopsy of the small intestine of the horse is indicated to investigate such conditions as IBD, neoplasia and Grass Sickness (Schumacher *et al*. 2000). The small intestine consists of a number of histological layers of intestine namely the inner mucosal layer, the submucosa, the muscularis and the outer serosal layer (Dyce *et al*. 2002).

One of the most common indications for FTIB in horses is in the diagnosis of Grass Sickness (Equine Dysautonomia), the histological areas of interest are the submucosal and myenteric ganglia i.e. the submucosa and outer muscular layer (Barlow 1969; Gilmour 1973; Uzal *et al*. 1994; Milne *et al*. 2005a; Milne *et al*. 2005b; Wales and Whitwell 2006; Waggett *et al*. 2009). A histopathological diagnosis of Grass Sickness is based on chromatolysis (the only parameter to be of a suitable sensitivity and specificity) in neurons of the myenteric plexus from an ileal biopsy (Scholes *et al*. 1993a, 1993b). The central neuronal changes were described as being degenerative in character, involving initial central chromatolysis and eccentric, pyknotic nuclei (Barlow 1969; Gilmour 1973; Wright and Hodson 1988). In the normal horse small submucosal ganglia (clusters of up to four neurons) lay close to the muscularis mucosae, whereas larger ganglia were situated more deeply near the circular muscle. Myenteric ganglia are usually located between the outer circular and innermost longitudinal muscle (Scholes *et al*. 1993a, 1993b). Synaptophysin is a synaptic vesicle glycoprotein present in neuroendocrine cells and in virtually all neurons in the brain and spinal cord that participate in synaptic transmission. Its ubiquity at the synapse has led to the use of synaptophysin immunostaining for quantification of synapses (Calhoun *et al*. 1996). It accumulates in degenerating neurons and can improve the accuracy of diagnosis of EGS (Waggett *et al*. 2010). A personal

communication with Prof. Milne of Edinburgh University stated that a biopsy site of 10-20cm proximal from the ileocaecal fold is adequate for Grass Sickness diagnosis. Furthermore, in a more acute case a more distal biopsy site is recommended.

The diagnosis of neoplasia can be most often achieved by a FTIB sample of the affected portion of intestine. Full thickness biopsies of bowel wall and mesenteric lymph nodes can be used to diagnose neoplastic bowel diseases. Bowel wall biopsies should be taken from several sites along the intestinal tract and at sites where macroscopic lesions are visible at exploratory surgery (Fischer 2002). Small intestine was found to be the most common section of intestine with neoplasia in a study of 34 horses divided into groups as follows - 19 Lymphoma, 11 Adenocarcinoma, 3 Leiomyosarcoma, 1 Leiomyoma (Taylor *et al*. 2006). Alimentary lymphoma is a disease, which may be a primary neoplastic disease, or may represent part of a multicentric disease or a metastatic spread from a primary focus somewhere else in the body (Platt 1987, 1988; Mair *et al*. 2006; Taylor *et al*. 2006). The two most predominant types of neoplasia i.e. lymphoma and adenocarcinoma require histological investigation of the submucosal and mucosal layer for diagnosis (Taylor *et al*. 2006). The disease may take the form of discrete focal tumour masses in the intestinal wall or a diffuse intestinal infiltrate of neoplastic cells that may cause malabsorption (Platt 1987, 1988). Both the small and/or large intestines may be affected, and malignant cells also commonly infiltrate mesenteric lymph nodes. Adenocarcinoma is a malignant tumour that can occur in the small intestine, caecum, and large colon (Honnas *et al*. 1987). The tumour arises from the glandular crypts of the gastrointestinal tract and was reported in middle-aged and older horses (Mair *et al*. 2006; Taylor *et al*. 2006). Leiomyosarcoma is a malignant tumour of the smooth muscle lining the gastrointestinal tract and has been reported in the stomach, small intestine, and rectum. Leiomyoma is a benign tumour of the smooth muscle of the gastrointestinal tract that can occur in the stomach small intestine and small colon (Johnson and Steinberg 1989; Mair *et al*. 1990; Bailey *et al*. 2003; Schaudien *et al*. 2007). Squamous cell carcinoma is a malignant tumour of the gastrointestinal tract and is the most common tumour of the oral cavity, oesophagus and stomach (Olsen 1992; McKenzie et al. 1997; White et al. 2008).

IBD can be diagnosed primarily by examination of the submucosal and mucosal layers of the intestine of the affected portion of intestine (Schumacher *et al*. 2000b; Packer *et al*. 2005; Ali and Tamboli 2008). Exploratory laparotomy and multiple full thickness bowel wall biopsy may be the only way to obtain a definitive diagnosis in the living horse with intestinal maldigestion and malabsorption (Mair *et al*. 2006). Chronic IBDs are associated with dysfunction of the gastrointestinal tract due to infiltration of the mucosa and submucosa with populations of eosinophils, plasma cells, lymphocytes, basophils, or macrophages (Schumacher *et al*. 2000b; Roberts 2004; Packer *et al*. 2005; Ali and Tamboli 2008). IBDs of horses either have a proven aetiology, e.g. mycobacterial (Rook *et al*. 2004; Ali and Tamboli 2008), toxic (Mair *et al*. 2006) or parasitic (Jasko and Roth 1984), or are idiopathic. IBD of the horse can be subdivided into 4 conditions; GE, LPE, EC and MEED. Clinically, they all present in a similar fashion, i.e. weight loss, usually with diarrhoea. However, associated skin lesions are reported in GE (11% cases) and MEED (63% cases) (Schumacher *et al*. 2000) and histological examination is required for a definitive diagnosis. The terms idiopathic EC and MEED are both terms to describe disease characterized by infiltration of intestinal and extraintestinal tissues with eosinophils. Chronic eosinophilic infiltrates may take the form of diffuse inflammatory cell infiltration of the small intestinal mucosa with eosinophils and lymphocytes or an eosinophilic granulomatous infiltrate (Gibson and Alders 1987; Schumacher *et al*. 2000b). Mucosal ulceration, enlargement of ileal Peyer's patches, and mesenteric lymphadenopathy are frequently present. The aetiology of the condition is unknown, but the nature of the inflammatory infiltrate has led to the suggestion that it represents an immune-mediated response to parasites (Perez Olmos *et al*. 2006). EC may occur as a diffuse infiltrative disease of the small intestine, or, more commonly, as a focal infiltrative lesion. In the small intestine, the latter often cause circumferential mural bands that cause a partial obstruction of the intestinal lumen, and are associated with colic (often recurrent colic) (Schumacher *et al*. 2000a; Southwood *et al*. 2000; Archer *et al*. 2006; Perez Olmos *et al*. 2006). Circumferential mural bands were considered to be pathognomonic for IBD and were thought to result from stimulation of fibrous connective tissue by enzymes elaborated by eosinophils (Mair *et al*. 2006). They are characterised histologically by mural infiltrates of eosinophils (Scott et al. 1999). Segmental eosinophilic colitis is an uncommon disease that results in a

local obstructive lesion of the left colon wall and resultant mild to moderate intermittent colic signs. Affected segments of intestine show variable mucosal necrosis, submucosal oedema, and eosinophil infiltration of the lamina propria and deeper layers of the colon wall (Gibson and Alders 1987; Schumacher *et al*. 2000b; Southwood *et al*. 2000; Mair *et al*. 2006). Multisystemic eosinophilic epitheliotrophic disease (MEED) is a chronic progressive multifocal disease affecting organ systems containing glandular epithelium, the target tissue for eosinophilic infiltration (Nimmo Wilkie *et al*. 1985). Horses affected with MEED can present with a variety of clinical signs, depending on the degree of involvement of these systems. Only two cases have been reported in the UK (Hillyer and Mair 1992; Henson *et al*. 2002). Lesions observed during histologic examination of rectal mucosa obtained by biopsy correlated with the post-mortem diagnosis in 6 of 12 horses affected with MEED. The presence of eosinophilic infiltrates in rectal mucosa and submucosa of normal horses is a common and insignificant finding, however the presence of eosinophilic granulomas, associated with vasculitis and fibrinoid necrosis of intramural vessels, is considered diagnostic of MEED (Zimmel 2004). GE is characterised by diffuse granulomatous lesions, predominantly in the small intestine, villous atrophy with lymphoid and macrophage infiltration of the mucosal lamina propria, and variable numbers of plasma cells and giant cells (Meuten *et al*. 1978; Woods *et al*. 1992). LPE is characterised by mucosal infiltration by lymphocytes and plasma cells in the absence of granulomatous change (MacAllister *et al*. 1990; Kemper *et al*. 2000). In other species, such as the dog, lymphocytic/plasmacytic enterocolitis is thought to represent a nonspecific intestinal immune response to agents that cause intestinal damage. It may represent a pre-lymphomatous change in such species; it is currently unknown if a similar progression may occur in the horse (Mair *et al*. 2006; Rowe and White 2008).

Enteric infections such as Mycobacterial granulomatous enterocolitis (Perdue *et al*. 1991), enteric fungal infections due to *Aspergillus fumigatus* or *Histoplasma capsulatum*; (Slocombe and Slauson 1988), *Lawsonia intracellularis* the obligate intracellular organism which is the cause of equine proliferative enteropathy (Pusterla and Gebhart 2009), parasitic infections (Cyathostominosis among other

rarer intestinal disease), and intestinal fibrosis (Traub-Dargatz *et al*. 1992; Schultheiss *et al*. 1995) may be diagnosed by FTIB.

The proposed rationale for excisional biopsy from midway between the mesenteric and anti-mesenteric border is to help avoid inclusion of a Peyer's Patch (PP) which would not offer an optimal sample for histopathological diagnosis (Mair *et al*. 2002). Lymphoid nodules, usually solitary are found through out the gastrointestinal tract both in the lamina propria and the submucosa. In the young horse PP are usually not apparent grossly in the stomach and small intestine, but are obvious in the caecum and large intestine. After about 1 year of age all lymphoid tissue aggregations are smaller and the nodules are no longer apparent grossly in the large intestine. Peyer's patches are infrequently seen grossly and histologically in the horse and are not as well developed as in other species, being essentially loose aggregations of solitary lymphoid nodules (Mair 2002). Intestinal glands within lymphoid nodules in the submucosa, common in cattle and swine, are rarely evident but occasionally occur in the submucosa of the apex of the caecum. There are more extensive infiltrations documented in pigs, sheep and lambs, dogs, and calves (Kararli 1995).

Techniques and indications to obtain full thickness intestinal biopsy in human beings

Similar to horses, there are a variety of gastrointestinal disorders in human medicine in which a specific tissue diagnosis markedly alters the course of treatment. The mid-small bowel is relatively inaccessible to the endoscopist. In human medicine samples of mucosa and submucosa are the most commonly performed intestinal biopsy achieved using endoscopic biopsies and suction biopsies; the complication rate is low and it is regarded as a routine procedure (King *et al*. 2005). Generally the attainment of FTIB is carried out by a laparoscopic assisted exteriorisation of intestine; excision of the FTIB was then carried out using conventional surgical instruments in the described way (Tjandra 2006). In a study of 124 patients who underwent laparoscopic FTIB with a clinicophysiological diagnoses of chronic intestinal pseudo-obstruction, enteric dysmotility or severe irritable bowel syndrome resulted in overall specific diagnostic yield of 81%, being high for jejunal biopsies (89%) but low for a small

number of ileal and colonic biopsies. No mortality and minimal morbidity was reported. It was concluded that laparoscopic assisted FTIB of the bowel appears acceptable in terms of safety (Knowles *et al*. 2008).

Hirschsprung disease (HD) and Cystic Fibrosis colonopathy are amongst the diseases where FTIB is required (Mazziotti and Langer 2001). In some of these cases, endoscopically obtained mucosal biopsies are not adequate, and a full-thickness bowel biopsy may be required (Mazziotti and Langer 2001). Retention of a proximal aganglionic segment or the unrecognized coexistence of other dysganglionoses may jeopardize the definitive surgical treatment of HD. The diagnosis of HD should take place early in the neonatal period, because without an effective diagnosis and appropriate treatment, a considerable proportion of infants will go on to develop serious complications such as acute enterocolitis or toxic megacolon (Meijer *et al*. 2004). Thus to assess the extent of the disease and/or the presence of other dysganglionoses without an additional laparotomy would be beneficial and a laparoscopic-assisted technique to perform colonic full-thickness biopsies by exteriorisation of the colon was developed (Carvalho *et al*. 2002). Colonic dysmotility is better analysed by examination of the colonic muscle than rectal biopsy, which does not examine the defective area and has a low yield; seromuscular biopsy of the colon was achieved by laparoscopic excision using an Endoshears® (Ethicon Endo-Surgery)[1] (Alexander *et al*. 2003). Intestinal lymphomas encompass those lymphomas with a dominant or only localized occurrence in the intestinal tract. Coeliac disease is highly associated with enteropathy-associated T-cell lymphomas. Coeliac disease-related lymphomas can appear at nodal or extranodal sites. This enteropathy is often multifocal with ulcerative lesions, which explains the high perforation rate at presentation or during chemotherapy. A thorough examination is required in identifying these conditions. Accurate diagnosis based on endoscopic or full thickness laparoscopic small-bowel biopsies are mandatory (Eltringham *et al*. 1993). HD is one of the most difficult diagnoses in paediatric surgery

[1] Ethicon Endo-Surgery®, Route 22 West Somerville, NJ 08876 USA

(Martuccielo 2008). Laparoscopic-assisted mapping of the entire colon was described and is a simple, safe, and effective procedure that may contribute to improving the outcome of intestinal dysganglionosis by better characterization of the HD (Carvalho *et al*. 2001). Full-thickness laparoscopic biopsy and a course of steroids might avoid a laparotomy in patients with the rare condition of subacute bowel obstruction related to eosinophilic gastroenteritis (Nagle *et al*. 2004).

Eltringham *et al*. (1993), Greig *et al*. (1995), and Mazziotti (2001) reported techniques, which permitted FTIB without the need for the traditional ventral midline laparotomy. The method is simple, permits full laparoscopic examination of the abdominal contents and being minimally invasive, facilitates early patient recovery (Schambourg and Marcoux 2006). Both Greig *et al*. (1995) and Mazziotti and Langar (2001) described techniques in human surgery and more recently the technique described by Bracamonte *et al*. (2008) in horses has involved the use of laparoscopic linear stapler to excise a fold of intestine. These are solely laparoscopic techniques and are performed completely intracorporeally.

There are also indications in human medicine where it is beneficial to obtain FTIB over mucosal biopsy for research purposes. For example laparoscopic assisted FTIB have been used to investigate the histopathologic functional bowel disorder irritable bowel syndrome and concluded that inflammation and neuronal degeneration in the myenteric plexus are involved in the pathogenesis of the disease (Tornblom *et al*. 2002; Tornblom *et al*. 2007). Further research using FTIB investigated the involvement of enteric neuropathy with mild inflammation (ganglionitis) associated with several motility disorders including irritable bowel syndrome, enteric dysmotility, slow-transit constipation and chronic intestinal pseudo-obstruction (Tornblom *et al*. 2007).

The techniques described using a linear stapler are not widely practised in human or veterinary medicine. The reasons for this are unknown but perhaps it is due to the perception that a large section of intestine is being excised and thus has the potential to cause a luminal stenosis (Evans *et al*. 2006). Furthermore it is conceivable that surgeons perceive that the placement of a linear stapler is difficult by the aid of the two dimensional information available

on a video screen and favour the more traditional and familiar technique of exteriorisation (Durrani and Preminger 1995; Regan and Guyer 1997; Maresceaux *et al*. 2002; Renda and Vallancien 2003).

Full thickness intestinal biopsy excision techniques in veterinary surgery

Until the recent techniques described by Schambourg and Marcoux (2006) and Bracamonte *et al*. (2008) either ventral median celiotomy or flank laparotomy or laparoscopic assisted flank laparotomy were the only reported techniques for obtaining full-thickness intestinal biopsies in horses (Bracamonte *et al*. 2008; Schambourg and Marcoux 2006). A laparoscopic assisted technique was first described by Eltringham et al. (1993) and was subsequently used in dogs. It is suitable for both FTIB and placement of a feeding jejunostomy tube; the intestine was exteriorised through a small 1cm laparoscopic portal sized incision to allow for the biopsy sample to be excised in a routine fashion from the antimesenteric border (Carvalho *et al*. 2002, Barnes *et al*.2006; Mayhew 2009; Rawlings *et al*. 2002).

There are other techniques, which have been described in dogs. This includes the technique where the jejunum was isolated extracorporeally and a no. 6 Keyes biopsy punch[2] was used to obtain a full-thickness sample from the anti-mesenteric surface of the jejunum (Keats *et al*. 2004). The defect was then closed in a transverse direction using *4-0* polydioxanone with a swaged-on taper needle in a simple interrupted appositional pattern. The depth of the cut on the Keyes biopsy instrument is limited to the blade length (approximately 8mm), but, theoretically, injury could occur to the opposite side of the bowel if too much force is applied to the punch or if the bowel wall is extremely thin. Additionally, in smaller dogs (and perhaps all cases), a smaller sized punch (i.e. 4mm instead of the 6-mm size) may be more appropriate. The 4-mm punch was

[2] Sklar Tru-punch®; Westchester, PA 19382, USA

routinely used by Keats *et al*. (2004) to obtain intestinal biopsies in cats. A vessel-sealing device can also be used to harvest a biopsy sample. A recent study compared use of the harmonic scalpel device[1] with the placement of haemostatic clips to harvest jejunal biopsy by a laparoscopic assisted technique (Barnes *et al*. 2006*)*. No haemorrhage was observed at harmonic scalpel biopsy sites of the stomach and jejunum whereas haemorrhage occurred in all dogs at the standard biopsy sites of the same organs. The harmonic scalpel led to a reduction in haemorrhage but resulted in significantly greater adhesion formation compared to a standard biopsy technique for biopsy of the jejunum (Barnes *et al*. 2006). Some degree of cellular damage occurs with this excision technique, however determination of the cells at the margin as benign or malignant was still possible (Metternich *et al*. 2002; Barnes *et al*. 2006; Phillips *et al*. 2008).

Laparoscopic exploration and subsequent exteriorization of loops of small intestine for biopsy through a small flank incision is the technique described in the standing horse (Fischer 2002). A 4-0 stay suture on a round body needle is placed. A number 11 blade is used to incise the intestine around the stay suture, ensuring that an adequate sample of mucosa as well as submucosa and muscularis is harvested. The incision can be closed using 3-0 or 4-0 monofilament absorbable suture material (e.g. polydioxanone) in a simple interrupted or simple continuous suture pattern. Schambourg and Marcoux (2006) collected full-thickness equine intestinal biopsies intracorporeally in a 2-step fashion. A horizontal elliptical biopsy, 7–10mm long and 7mm wide, was made with laparoscopic scissors through the serosa and the muscularis, leaving the mucosa intact. The intestinal seromuscular biopsy was removed through portal 3, and the partial thickness incision was then partially closed using a Lembert pattern (3-0 lactomer 9-1 with a ski jump needle); Polysorb®[3] and a PDS knot substitute (Lapra-Tyt®, XC- 200[1]) was fixed at the end. During that step,

[3] ESK3, Syneture Inc., Norwalk, CT, USA

care was taken to ensure that the mucosa and submucosa were everted throughout the non-sutured portion of the incision by periodically applying tension on the mucosa with the tip of the Kelly forceps. The mucosa–submucosa was then biopsied by cutting the tissues with scissors and retrieved, then the last stitch of the continuous suture was made and the suture tightened. Tension was applied when closing the suture with application of a 2nd Lapra-Tyt® knot substitute (Schambourg and Marcoux 2006).

A procedure using an endoscopic linear stapler to obtain FTIB of the jejunum was described by Greig *et al*. (1995) and Mazzioti and Langar (2001) in human beings and similarly in a small number of horses by Bracamonte et al. (2008). An endoscopic linear stapler uses a cutting knife which is advanced through the centre of two double rows of staples which are actuated by pusher blocks contacting wedges so that the incision is closed before the incision is made (Olson *et al*. 1994). Greig *et al*. (1995) describes the technique in human beings as the placement of a laparoscope through the umbilicus and subsequent placement of a 5mm cannula in the epigastrium and midline. The abnormal intestine is grasped by atraumatic forceps and held up towards the anterior abdominal wall. Care is needed to ensure that only one wall of the bowel is grasped. The 5mm cannula is replaced with a 15mm cannula and an Endo GIA 60 stapler®[4] is passed and placed transversely across the antimesenteric border to reduce the risk of stenosis and fired, releasing a FTIB specimen which is removed with atraumatic forceps. Mazzioti and Langer (2001) described a similar technique in the small bowel, colon and stomach in human beings however a 12mm cannula was used and the type of linear stapler was not described. One of the potential concerns using this approach is narrowing of the lumen at the staple line (especially in the small bowel). Narrowing of the bowel was avoided

[4] Autosuture®, Covidien plc., 20 Lower Hatch Street, Dublin 2, Ireland

by placing the stapler transversely across the anti-mesenteric surface of the bowel (Mazziotti and Langer 2001). In small animal surgery biopsies of the small intestine are usually performed using a laparoscopic-assisted technique as the linear stapler technique is perceived to be more likely to cause luminal stenosis (Evans et al. 2006). In the study by Bracamonte *et al*. (2008) a 45-mm endoscopic articulating linear stapler with a 440-mm shaft was used to collect biopsy specimens - long 45A Endocutter ETS-Flex45® [1]. The endoscopic linear stapler cartridges contained four staggered rows of titanium staples with a leg length of 4.1mm (Reloads 4.1-mm ETS45, Endopath® [1]). To collect the biopsy specimens, the open jaws of the ELS were applied on the anti-mesenteric border of the middle section of the intestinal segment at a 10° angle to its long axis. The jaws were closed and the device fired to both activate the four parallel staggered rows of staples and make a full-thickness section through the intestinal wall, leaving 2 staggered rows of titanium staples on both the biopsy specimen and the biopsy site. The stapler was reloaded, and a second cut was performed crossing the first one at a 120° angle to obtain a full-thickness V-shaped intestinal biopsy specimen Bracamonte *et al*. (2008).

FTIB of the rectum obtained at post mortem was investigated in the diagnosis of Grass Sickness (Wales and Whitwell 2006). FTIB samples were obtained in this study and not rectal biopsy using a grasping forceps as is commonly performed in practice. Although the techniques described by Wales and Whitwell (2006) need to be evaluated with tissues from live cases in practice, they do appear to be of value in helping to provide an ante mortem diagnosis of Grass Sickness, although they would probably be less useful when attempting to rule out the condition given that they have a sensitivity of 71% and a specificity of 100%.

Size and orientation of biopsy

Scholes *et al*. (1993) used a trans-mural elliptical shaped ileal biopsy from midway between the antimesenteric and mesenteric border excised at the proximal end of the ileocaecal fold measuring 10mm to 15mm longitudinally. The two-stage biopsy laparoscopic technique (seromuscular, submucosa–mucosa) as described by Schambourg and Marcoux (2006) resulted in a 7mm-10mm elliptical excision; biopsies submitted for histopathologic evaluation were of

good diagnostic quality, adequate size and had minimal crushing artefact. Bracamonte *et al*. (2008) described the biopsy site as a 10mm by 30mm elliptical incision midway between the anti-mesenteric and mesenteric borders of the descending duodenum and distal jejunum (Schumacher *et al*. 2000b). Keats *et al*. 2004 used a No. 6 punch (circle shape and 6mm in diameter) biopsy to take small intestinal samples from the anti-mesenteric portion of only one wall of the jejunum in dogs and reported no differences in speed of collection, diagnostic value of specimens, complication rates or sample quality as compared to a standard excision protocol. In small animals, removal of full-thickness intestinal biopsy specimens larger than 20% of the luminal diameter may cause stricture (Willard *et al*. 2001; Willard *et al*. 2002) and >33% reduction of luminal diameter at an intestinal anastomatic site is potentially dangerous in horses (Reinertson 1976). The biopsy obtained by Bracamonte *et al*. (2008) reduced luminal diameter by 21.5% without a related post-operative complication. The degree of luminal diameter reduction must be balanced against the achievement of an adequately sized biopsy to enable a pathologist to make a correct interpretation and yield an accurate diagnosis (Willard *et al*. 2002).

Closure of the biopsy excision site

Laparoscopic procedures remain highly technical, requiring specialized and expensive instrumentation as well as considerable training to decrease the risks associated with the procedure, especially intracorporeal suturing (Chamness 2002). General complications reported with intestinal biopsies and small intestinal surgery are dehiscence, luminal strictures, adhesions and post operative ileus (Bellenger 1982; Ellison 1989; Orscher and Rosin 1993; Thornton and Barbul 1997; Freeman 2009a). Many of the complications are directly related to the effectiveness of the BES closure and the complications can have dire consequences. For example 12% mortality in dogs following routine incisional biopsy in a series of 66 dogs was reported (Shales *et al*. 2005). Lower or no complication rates have been cited elsewhere (Evans *et al*. 2006; Schambourg and Marcoux 2006; Keats *et al*. 2004 and Bracamonte *et al*. 2008). Endoscopic stapling devices have gained widespread clinical use. In human beings, use of endoscopic staplers for obtaining laparoscopic full thickness biopsy specimens is safe and effective (Greig *et al*. 1995; Mazziotti and Langer

2001). Applying traction on the anti-mesenteric border of the small intestine with a laparoscopic forceps and firing the endoscopic linear stapler across the tented anti-mesenteric border of the intestine was used to collect intestinal biopsy specimens. This technique decreases the risk of postoperative stenosis (Greig *et al*. 1995; Mazziotti and Langer 2001). In the Bracamonte et al. (2008) study, equine biopsy specimens were collected by applying 2 crossing staple lines to obtain a V-shaped biopsy specimen from the anti-mesenteric border. The authors felt that it was not possible to apply the staples across the intestine in a transverse manner as it was felt that the short mesentery of the duodenum prevented this.

In horses, use of automatic stapling devices reduces surgical time and risk of contamination, compared with conventional hand-sutured anastomosis techniques (Baxter *et al*. 1992a; Mueller and Allen 1996). Skin staples were used to perform anastomosis of small intestine in the horse and dog (Ang *et al*. 1998; Coolman *et al*. 2000; Gandini and Bertuglia 2006). Auto-suturing and stapling devices have been developed to facilitate suturing e.g. Suture Assistant™, Lapara-Ty™, Multifire Endohernia®[1]. Auto-suturing devices most often have straight needles, which are too short for a thickened wall and make partial thickness suturing cumbersome, as the angle of tissue penetration is extremely difficult to adjust. Because the application head is in a fixed position they are less versatile as tissue alignment with the laparoscope portal is critical. Others have concluded that these devices were not suitable for accurate suturing (Baxter *et al*. 1992b; Bracamonte *et al*. 2008). On the other hand, stapling devices greatly reduce suturing time but even the largest staples currently available (4.8mm) are too small for thickened intestinal walls, where they do not hold the intestinal submucosa together. This results in a lower bursting strength compared with a single layer, continuous pattern with a laparoscopic needle holder (Baxter *et al*. 1992a). Intracorporeal and extracorporeal suturing is technically challenging (Simpson 1974). The 4.8mm staple of the TA-90 Premium stapler[4] closes to an approximate size of 2mm (Blackford and Blackford 2006).

Laparoscopic instrument design

Technical aspects of instrumentation

Medical instruments should be a natural extension of the human body (Chamness 2002). Complexity in mechanism assembly increase the cost of manufacturing based on the increased number of parts, and the time spent in arriving at a suitable design (often through trial and error) (Lim and Erdman 2002; Lim and Erdman 2003).

Conventional solutions of mechanical design should be explored however are not always satisfactory. Awareness of forces helps to stimulate the design of simple and efficient mechanisms. For these reasons, it is more natural to start the design with the specification of profitable configurations of forces, or desired force transmission characteristics, rather than the usual specification of desired motion and conventional solutions. The approach of taking profitable configurations of forces as the point of departure is "force-directed design" (Grimbergen 1997).

The type synthesis methodology described by Lim and Erdman (2002) allows the designer the benefit of understanding the inter-relationships between each component of an instrument, which in turn helps define the overall purpose of the instrument. Thus the functions of an instrument can be divided into subsystems, for example excision, staple closure and design can be divided into specific steps to allow for progression of design through enumeration of possible solutions and a rational approach to designing and redesigning medical instruments. This design methodology leads to simpler, more reliable and effective surgical instruments (Lim and Erdman 2002). Similarly type synthesis methodology aims to identify the function and thus the forces required in the use of an instrument. By the identification and division of the desired functions, objectives, kinematics and design rules it allows for identification of similar solutions in previously designed instruments and thus aids in design solutions (Lim and Erdman 2002, Lim and Erdman 2003).

It is important to recognize all of the key requirements of an instrument early in its innovation and conception. The requirements are of the following three basic

types: functional, capabilities and constraint. It must also be recognised that the emphasis on certain requirements changes in the progression of the product development (Martin 1997). For example the colour of the instrument is not important in the developmental phase whilst it is in the commercial phase of the product when the emphasis is on marketing the product. A common mistake is to treat all requirements as equally important or assuming the wrong priority for a requirement (Martin 1997). In the development of a product one must recognise "the whole universe of the requirements". Firstly, the stakeholders must be identified at the conception stage of the instrument development and these are the end user or surgeon and the patient, the histopathologist and the engineers/manufacturers. Accurate understanding of user/stakeholders needs is the factor that discriminates most strongly between commercially successful industrial good innovation projects and those that fail (Sydenham 2004).

The instruments actions must be possible in an intuitive, comfortable and easy manner. For intuitive control, the surgeon's actions must correspond with actions of the instrument, e.g. exerting force on a hand-grip of a forceps corresponds with the pinching of the gripper (Lim and Erdman 2003). Feedback information must be supplied in such a way that forces and displacements are undisturbed and within the range of good sensitivity of the surgeon's hands. The instrument's acions should be physically (requiring an energy efficient design without play, friction etc.) and mentally (requiring an obvious operating principle) efficient, while the overall control scheme must correlate with human nature, being comfortable requiring good ergonomics throughout the handling procedure (Hogan 1985; Grimbergen 1997).

Laparoscopic equipment design and innovation (Lim and Erdman 2003).

Many surgical instruments consist of basic mechanical components such as gears, links, pivots, sliders, etc., which are common in mechanical design. Table 2-1 outlines the mechanical characteristic and the differences between 16 patented laparoscopic surgical devices, most of which are surgical staplers as described by Lim and Erdman (2003). This is beneficial in many ways for example, in explanation of design constraints and the mechanisms used in previous stapler

designs and their comparison, it allows exploration and selection of different components for specific output function(s), and it helps address possible intellectual property issues and to avoid reinventing ‘‘the wheel’’.

INPUT	Self Contained Power		Manual	
	Electric Motor	Pneumatic	Rotational	Sliding
Intermediate Mechanism	Gear trains	Gas cylinders-drive piston, mechanical slide, linkage	Rack and pinion, springs, ratchet, mechanical slide	Linkages, springs
OUTPUT				
Rotate	Rotatable collar	Rotatable collar	Rotatable collar	Rotatable collar
Yaw	None	Pulley, cables and linkage	Flexible links (Push/pull), cables/belts, pulleys	Flexible links (Push/pull), cables/belts, pulleys, spine segments
Clamping	Lead Screw sliders	Linkage and cables	Mechanical slide	Mechanical slide, cam springs
Eject Staples		Flexible Slide	Mechanical slide, cam, pulley, belt/cable, linkage, springs	Mechanical slide, cam, springs
Cutting				
DESIGN		Simple (less parts)		Complex
ACTIVATION			Minimal/no control (press a button/trigger)	Direct Control

Table 2-1 Comparison table for designing laparoscopic surgical stapler and instruments reproduced from Lim and Erdman (2003). The table outlines the different mechanisms used in the five stages of input i.e. Intermediate mechanisms, Output (Rotate, Yaw, Clamping, Eject Staples, Cutting, Design and Activation).

Each of the headings in Table 2-1 represents a major component of an instrument that will enable it to accomplish its required function(s). The inputs and outputs were not directly connected in any of the 16 surgical staplers investigated by Lim and Erdman (2003). The input or source of power for the instrument can be varied. Over automation of instrumentation is undesirable both because of the complexities of manufacturing and due to hazardous force generation and its unfamiliar interaction and feedback with soft tissue as opposed to what occurs in industrial applications. Force control or impedance control are more appropriate and essentially safer (van Leerdam and 1993;

Nijenbanning 1998). Therefore, body power is preferred over external force, such as electric or pneumatic power systems. A positive side effect of this approach is that less complex and lighter instruments result, without the burden of electric wires. Low friction mechanical solutions are required to make use of the feedback present. Alternatives for low friction designs are active friction (and inertia) compensation or teleoperation. These approaches allow easy adjustment of the transfer function, which may be useful, e.g. in situations where operating forces are below the human sensory threshold, such as in microsurgery however the economic and engineering inputs of these mechanisms outweigh their discussion in this thesis (Lim and Erdman 2002). Furthermore, to be a natural extension, instruments preferably should be fast, silent, lightweight and reliable (Lim and Erdman 2003).

The output heading is defined as rotate, yaw, clamp, eject, staple, and cut. These sub-categories are common movement options that were observed amongst the 16 patents investigated in the study. Most of them are designed into the surgical stapler for ergonomic reasons, so that surgeons can effortlessly use the device in a comfortable position. For example, to manipulate the stapler to the location where the tissue is to be treated, the surgeon can use the instrument's yaw, and/or capability for rotation. As part of the operation, the instrument must clamp onto the tissue then staple tissues together (top and bottom pieces of the tissue) and cut between the staples (for some instruments). The design category requires a qualitative measure; the number of mechanisms involved and the way they are used can be simple or complex. Simple refers to relatively fewer and less complicated mechanical components. The activation category refers to the control of input motion required to achieve the required output functions. In self-contained power, surgeons have little or no control over the output once the button or trigger is compressed. For manual input, surgeons can usually control how fast the output function is being performed, especially for ejecting the staples and cutting the tissue. By controlling the rate at which the trigger is squeezed (or other means of activation), the surgeon can determine how fast he/she wants the staples to be ejected and/or the tissue to be cut. The design challenge is to transform the movement of the surgeon's hand through a long small diameter trocar creating one or more output functions at the distal tip inside the body cavity. Matching

the characteristics of man and machine requires a comprehensive approach in which forces play a key role. Precise motions are often of less concern than matters such as stability, low force levels, force distribution, transmission and feedback. Mechanical design usually applies a kinematic perspective. Desired motion is taken as the point of departure for the type and dimension synthesis of a linkage (Lim and Erdman 2002).

Chapter 3. Material and Methods

Incorporation of the selected biopsy excision and closure technique within prototype instrument

The first limitation was the identification of the optimal excision shape, size and technique. The second limitation was the identification of an appropriate BES closure technique. These techniques were established in the preliminary investigations of the study (Appendix 4) and adopted for use in a FTIB laparoscopic instrument. All parts of the study were performed on post mortem specimens from horses euthanized for reasons unrelated to this study (Appendix 2) and the study was approved by the Animal Ethics and Welfare Committee of the School of Veterinary Medicine, University of Glasgow.

The prototype instruments were of a construction that could be adapted to a functional laparoscopic instrument. The standard laparoscopic portals used are conical in shape and their diameter range in size. However the largest commonly used in equine surgery is 15mm diameter (Fischer 2002b). The instrument must be powered from outside the abdomen i.e. at the end of a handle. The more complex the device the greater the cost; one of the aims of this project was to develop an instrument that was economical to produce. For this reason the input source to be investigated was chosen to be manual. The aim in the initial stage of the prototype investigation was the ability to excise a biopsy and thus the Prototype Instrument 1 and Prototype Instrument 2 focused on the ability to excise biopsy. The Prototype Instrument 3 incorporated an excision and closure method. The chosen methods were determined by the preliminary investigations (Appendices 3 and 4).

Prototype Instrument 1 – biopsy excision and gross assessment

Design, Description and Dimensions

The chosen input, intermediate mechanisms, output and activation are outlined (Table 3-1). The design specifications of the head of the instrument allowed room for a double layer of staples and for a biopsy excision (approximately 6mm

by 3mm) within the 15mm diameter are illustrated in Figure 3-1. The required specifications were estimated by an examination of a number of linear staplers. The length of the U-shape was enlarged to 7mm to allow for a margin of error in the manufacturing procedure. The diameter of the conical shaped instrument was designed to be 13.5mm; this allowed for approximately 1.5mm for the outer sleeve or supporting outer layer of the instrument (Figure 3-1).

Input	Manual
Intermediate Mechanisms	Mechanical screw plunger
Output	Biopsy Excision and Closure of BES
Rotate	The ability of the instrument to rotate in its current format is intricate to its cylindrical shape and symmetrical handle i.e. the handle in each of the prototypes was functional in all planes
Yaw	n/a
Clamping	Needle Stabilisation or the equivalent i.e. the legs of stables are required
Eject Staples	Advancement of outer plunger
Cutting	Advancement of the inner plunger and thus advancement of U-shaped blade to the dye on the head of the instrument
Activation	Direct Manual Control

Table 3-1 The comparison table for designing laparoscopic surgical staplers similar to Lim and Erdman (2003) adapted to outline the design of the excision instrument. (Prototype 1 and 2 do not avail of all the proposed features - Prototype 3 includes both a staple closure and U-shaped biopsy excision)

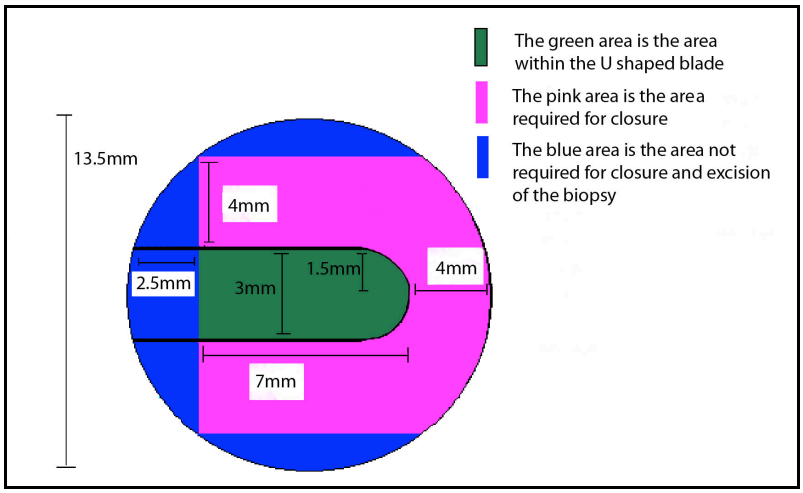


Figure 3-1 The cross sectional area of the proposed instrument. Approximations and extrapolations of measurements were made from investigation of the biopsy excisions and biopsy closures and from linear staplers.

All the Prototype Instruments were manufactured by CNC Blue Chip Engineering Ltd. The design drawings (Figure 3-11 to 3-15, 3-17, 3-19 to 3-22) were created using Premium® (Solidworks) and the presented illustrations were reproduced by SolidWorks e-Drawings® (Solidworks). The Prototype Instrument 1 was designed to trial the excision of biopsy. The instrument was used on the distal jejunum and ileum and a gross assessment of the biopsy carried out. No closure method was included in this trial.

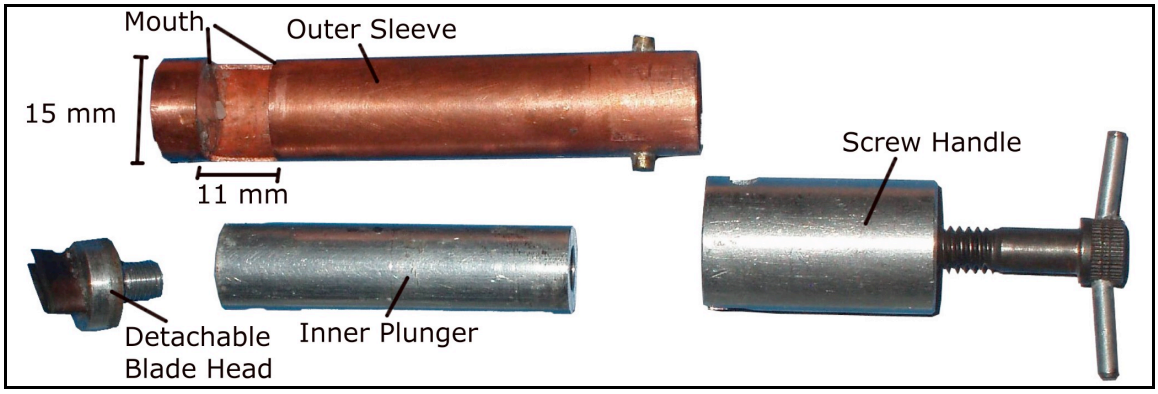


Figure 3-2 The components of the Prototype Instrument 1. The image is not to scale.

Outer Sleeve

The outer sleeve measured 8cm longitudinally and had a diameter of 15mm. The material used was copper. Copper rivets were attached to allow attachment of the screw handle. The mouth measured 11mm longitudinally and 9mm transversely. The dye on the head portion was a flat surface and was made of stainless steel.

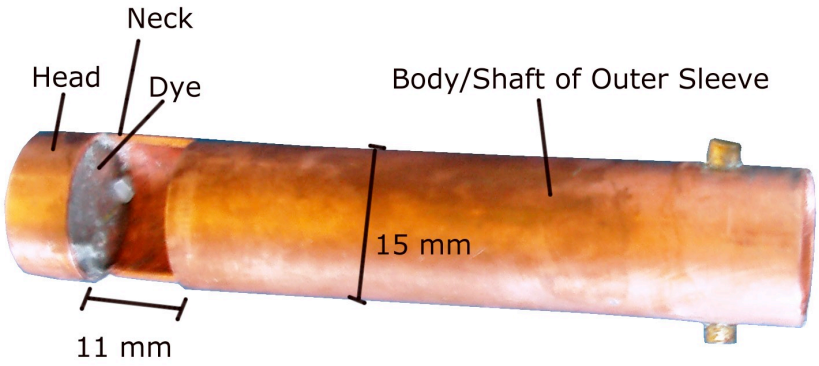


Figure 3-3 The outer sleeve of Prototype Instrument 1. The image is not to scale.

Detachable Blade and Plunger

The diameter of the detachable blade unit was 13mm. The U-shaped blade measured 12mm longitudinally, 3mm transversely and its height was 6mm. The U-shaped blades were attached to a face, which was attached to the plunger by a screw attachment. The plunger measured 5cm longitudinally and had a diameter of 13mm. Both the detachable blade and the plunger were made of stainless steel. The U-Shaped blade was made of a feather blade microtome blade (Surgipath®), which was manipulated into its U-Shape with the aid of a gas flame.

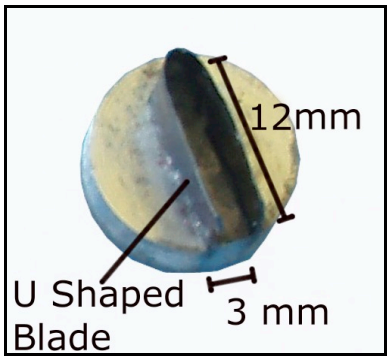


Figure 3-4 The detachable blade of Prototype Instrument 1. The image is not to scale.

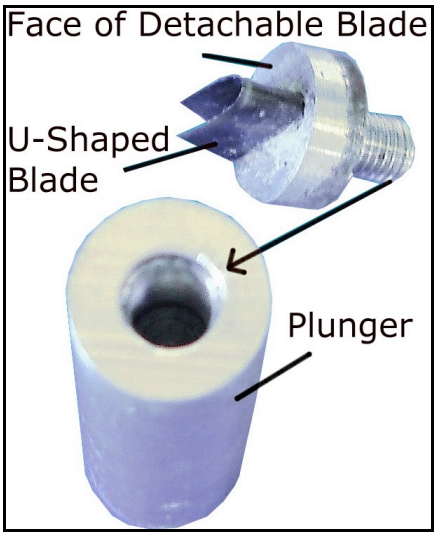


Figure 3-5 The detachable blade and its associated plunger. The image is not to scale.

Handle Attachment and Screw Turn Handle

The handle attachment and screw turn handle were made of stainless steel. The handle attachment encompassed the outer sleeve and was secured by copper rivets. The screw turn handle when rotated clockwise resulted in advancement of the inner plunger and detachable blade towards the dye on the head of the instrument.

Prototype Instrument 2 - biopsy excision and histological assessment

Design, Description and Dimensions

The aim of this part of the study was to evaluate Prototype Instrument 2 by a histological examination. Needle stabilisation was incorporated as part of this prototype instrument. The Prototype Instrument 2 was made of stainless steel and similar to Prototype Instrument 1 in that it consisted of an Outer Sleeve, Hand Held Plunger, a U-Shaped Blade on a detachable head with needle

stabilisation. Furthermore, the instrument's head was detachable by the aid of a screw attachment.

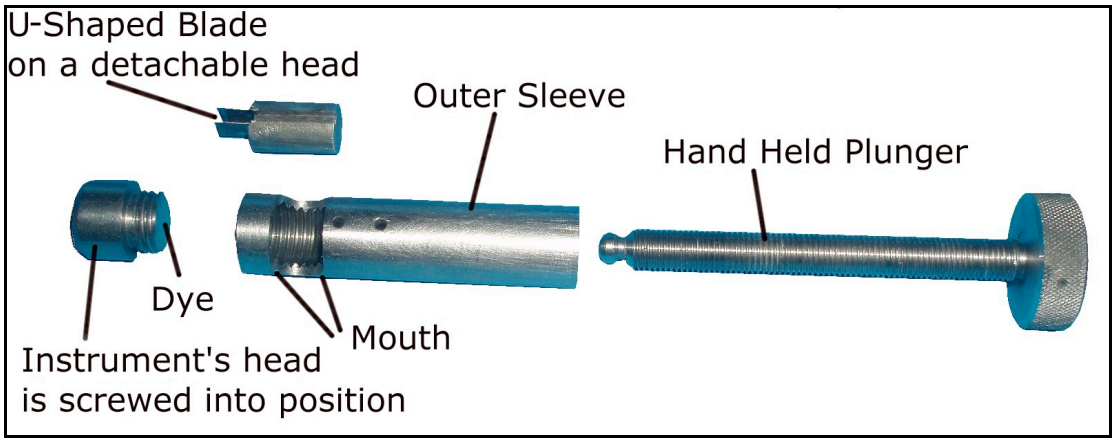


Figure 3-6 The components of Prototype instrument 2. The image is not to scale.

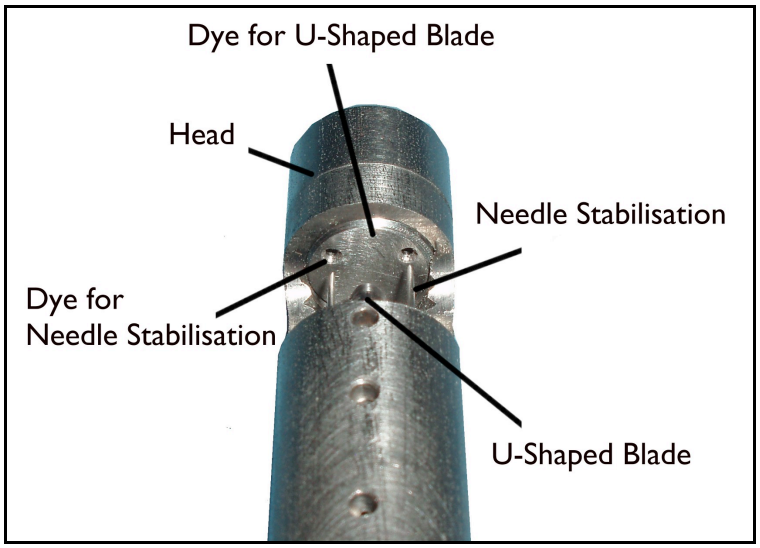


Figure 3-7 The Prototype Instrument 2 – the view is oblique from the bottom to the top; it shows the needle stabilisation. The image is not to scale.

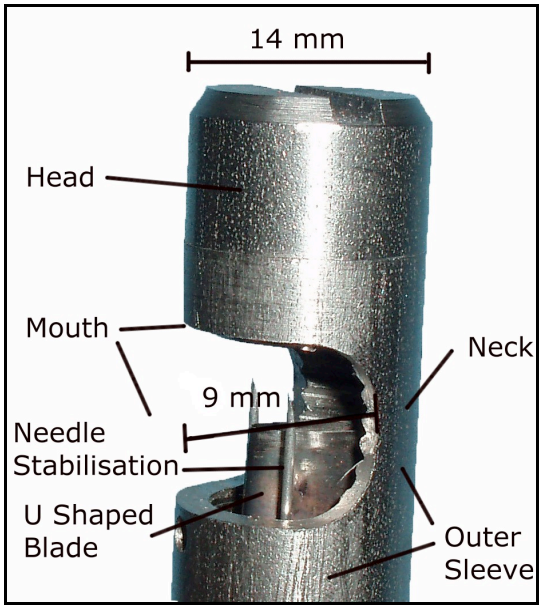


Figure 3-8 The Prototype Instrument 2 - The U-shaped blade and needle stabilisation have been advanced towards the head of the instrument by the plunger. The image is not to scale.

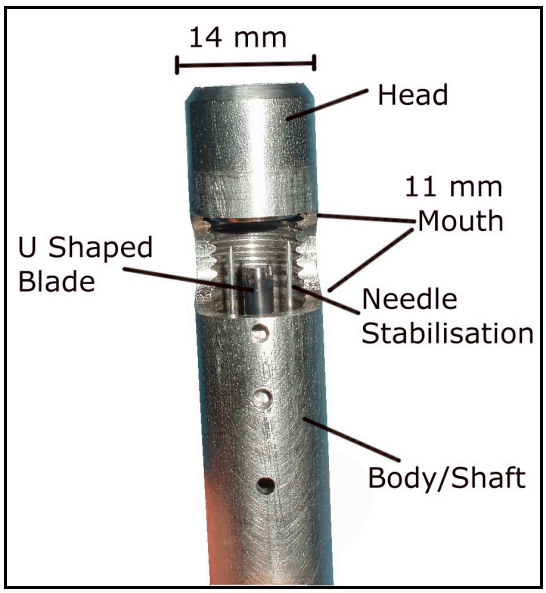


Figure 3-9 The Prototype Instrument 2 – a view from the front. The image is not to scale.

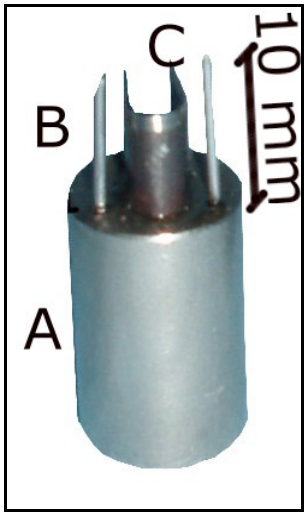


Figure 3-10 An enlarged image of the detachable blade. A = the body of the blade, B = the needle stabilisation mechanism, C = The U-shaped blade. The image is not to scale.

Outer Sleeve

The outer sleeve had a mouth that measured similarly to Prototype Instrument 1 (11mm longitudinally and 9mm transversely). The walls of the outer sleeve were 2mm thick.

Screw Handle and Plunger

The screw handle screwed into the base of the outer sleeve and thus the inner plunger and U-Shaped blade (on detachable head) advanced towards the dye and instrument's head, which served to power the excision of the biopsy.

Instrument Head, Dye area and Detachable U-Shaped Blade

The instrument's head in this prototype was detachable to enable samples to be retrieved easily. There were portals created in the dye area, which allowed for the needle stabilisation to enter as the inner plunger was advanced. The microtome blades fashioned into a U-shape similar to Prototype Instrument 1 and secured on stainless steel heads (See figure 3-10) were advanced towards the dye area by the plunger. The U-Shaped Blade was positioned more centrally than in Prototype Instrument 1 and measured 7mm longitudinally and 3mm transversely. The needle stabilisation consisted of two hypodermic needles (23 gauge) and each were 10mm in length.

Histological assessment

The biopsy obtained by Prototype Instrument 1 was assessed by grossly only whilst 30 biopsy samples obtained by Prototype Instrument 2 were submitted for histological assessment by an experienced histopathologist. Control FTIB comprised of the last fifteen clinical cases obtained from the distal jejunum and ileum of horses at the Weipers Centre Equine Hospital. These were obtained by the use of standard surgical instruments via either a laparoscopic assisted technique or a flank laparotomy or a ventral midline laparotomy. Each biopsy (Prototype Instrument 2 n=30 and Control Sample n=15) were attributed a biopsy score. An analysis using a 2 sample Wilcoxon rank sum (Mann-Whitney) test was performed. The details of the histological assessment are outlined in Appendix 3. Comments on the biopsy thickness, the mucosal and submucosal, the serosal and muscularis, myenteric and submucosal ganglia and the presence or absence of Peyer's Patch are summarised in the results chapter.

Prototype 3 - closure mechanism

Design, Description, Dimensions

The prototype instrument 3 included a closure method. It consisted of an outer sleeve, an inner plunger connected to the U-shaped blade, an outer plunger attached to individual staple plungers, a staple cartridge containing 10 staple cassettes. The head of the instrument consisted of a staple anvil and a cutting dye surface against which the U-shape blade was used to excise the biopsy. The closure method was evaluated by intraluminal bursting pressures and was compared to the intraluminal bursting pressures of the techniques evaluated in the preliminary study.

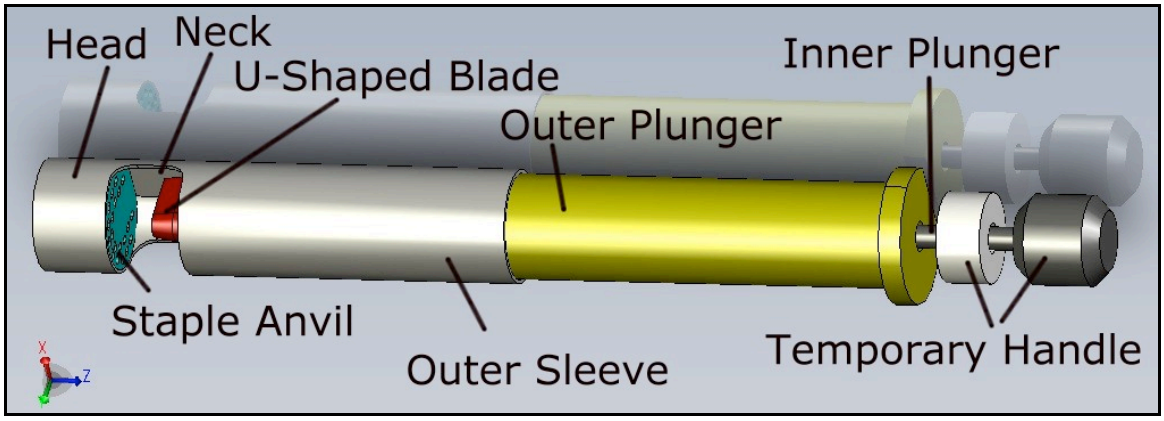


Figure 3-11 Design drawing: The components of Prototype instrument 3

Outer Sleeve

The outer sleeve was modified from the Prototype 2 Instrument. Its walls measured 0.07mm. The outer curvature of the outer sleeve was cylindrical. The inner curvature was cylindrical at the front of the instrument however the back wall of the instrument was reinforced by the creation of 1.1mm tangential back wall construct (Figure 3.13). This tangential construct spanned the length of the instrument and thus served to re-enforce the neck area of the instrument.

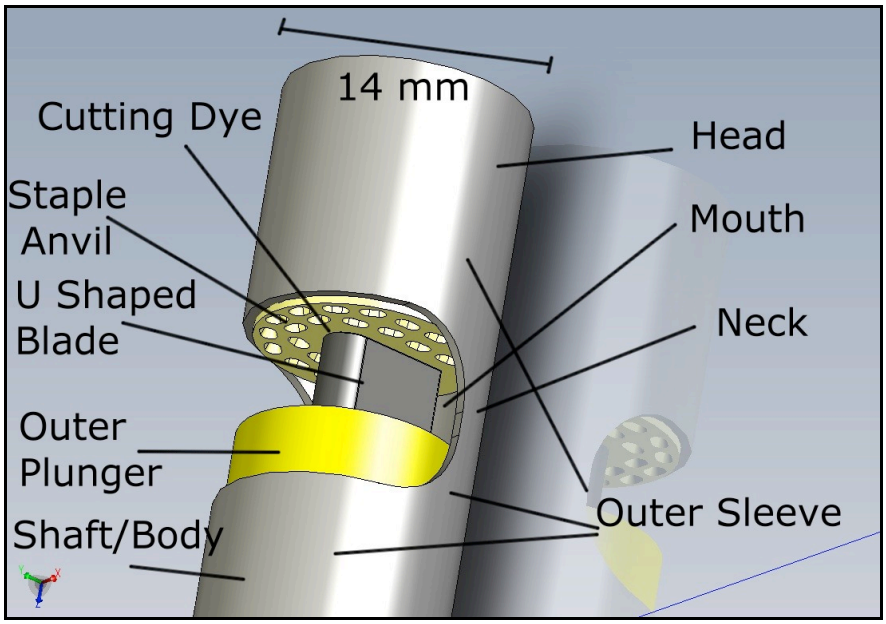


Figure 3-12 Design drawing: The head, neck, outer sleeve and outer plunger of Prototype Instrument 3

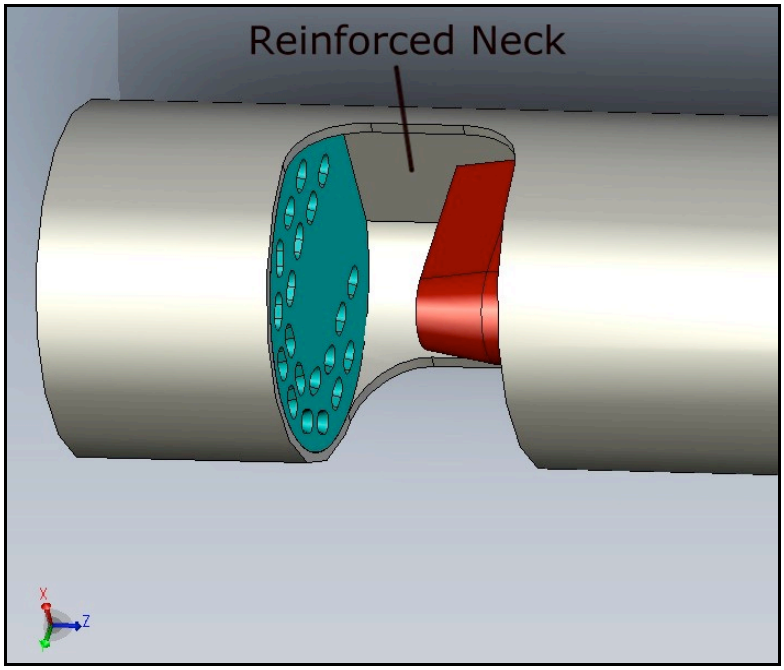


Figure 3-13 Design drawing: The reinforced neck of the outer sleeve of Prototype Instrument 3

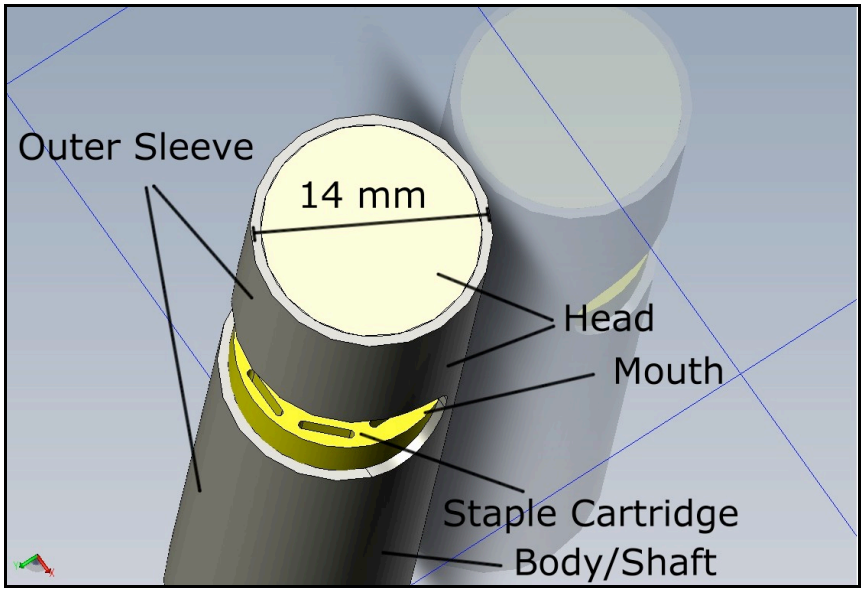


Figure 3-14 Design drawing: The head and outer sleeve of Prototype Instrument 3

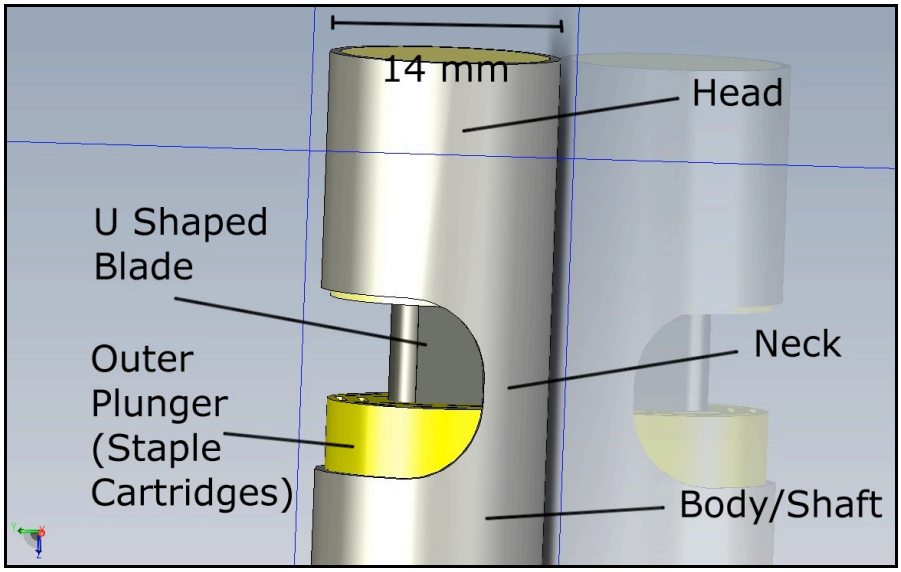


Figure 3-15 Design drawing: The side of the prototype instrument illustrating the mouth and neck of Prototype Instrument 3

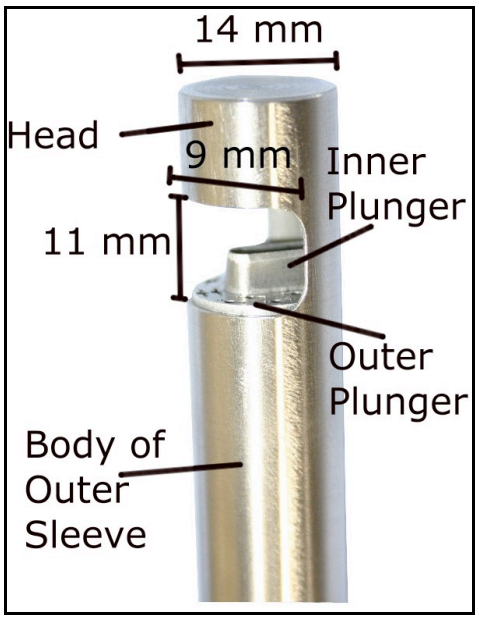


Figure 3-16 The head and mouth of Prototype Instrument 3. It is a side view of the instrument. The head, the inner and outer plunger, and the body of the outer sleeve and approximate measurements were denoted.

Outer Plunger, Staple Cartridge and Individual Staple Plungers

The outer plunger was defined as the construct encompassing the shaft of the outer plunger, the staple cartridge and the individual staple plungers. The staple cartridge consisted of ten staple cassettes. Each cassette had an individual staple plunger, which was attached to the shaft of the outer plunger. The staple

cartridge moved freely of the individual staple plungers. To load the staples the individual staple plungers were retracted i.e. the shaft was retracted approximately 5mm from the staple cartridge so as to allow room for the staples to load.

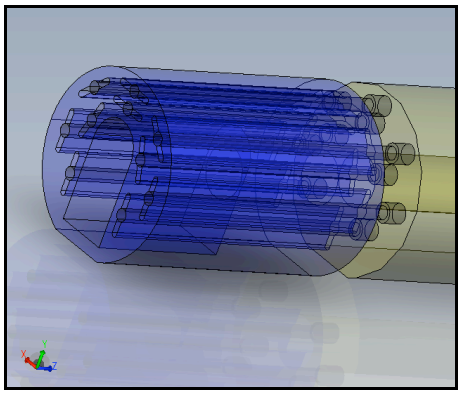


Figure 3-17 Design drawing: The outer plunger of Prototype Instrument 3. The layers are transparent allowing visualisation of the individual staple plungers and staple cassettes.

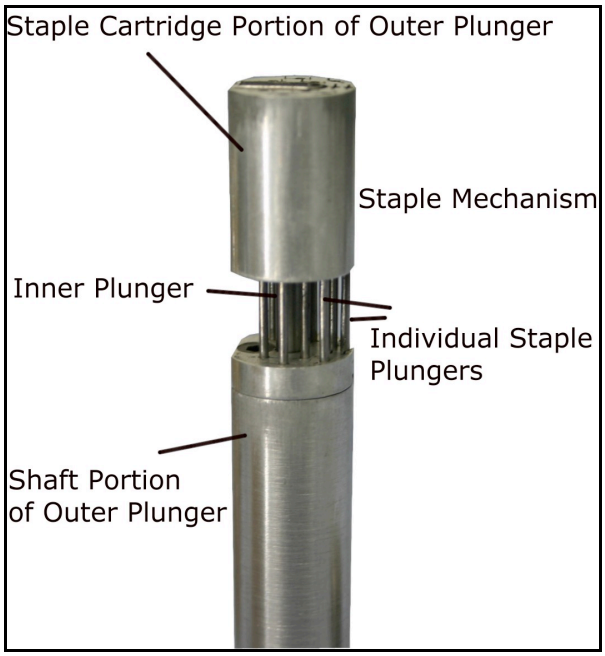


Figure 3-18 The shaft, individual staple plungers and staple cartridge portion of the outer sleeve of Prototype Instrument 3

Inner Plunger, U-Shaped Blade and Central Dye area

The inner plunger was attached to the U-Shaped blade and the inner plunger was within the outer plunger as illustrated in Figure 3-18. The U-shaped blade was

made of stainless steel and was manufactured specifically for this prototype. The U-shaped blade was advanced until the biopsy was excised similarly to previous constructs against the central dye area on the anvil.

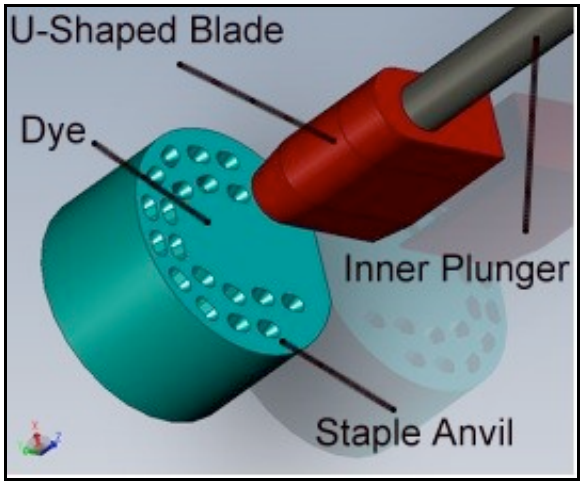


Figure 3-19 Design drawing: The inner plunger, U-shaped blade central dye and staple anvil of Prototype 3. The image is not to scale.

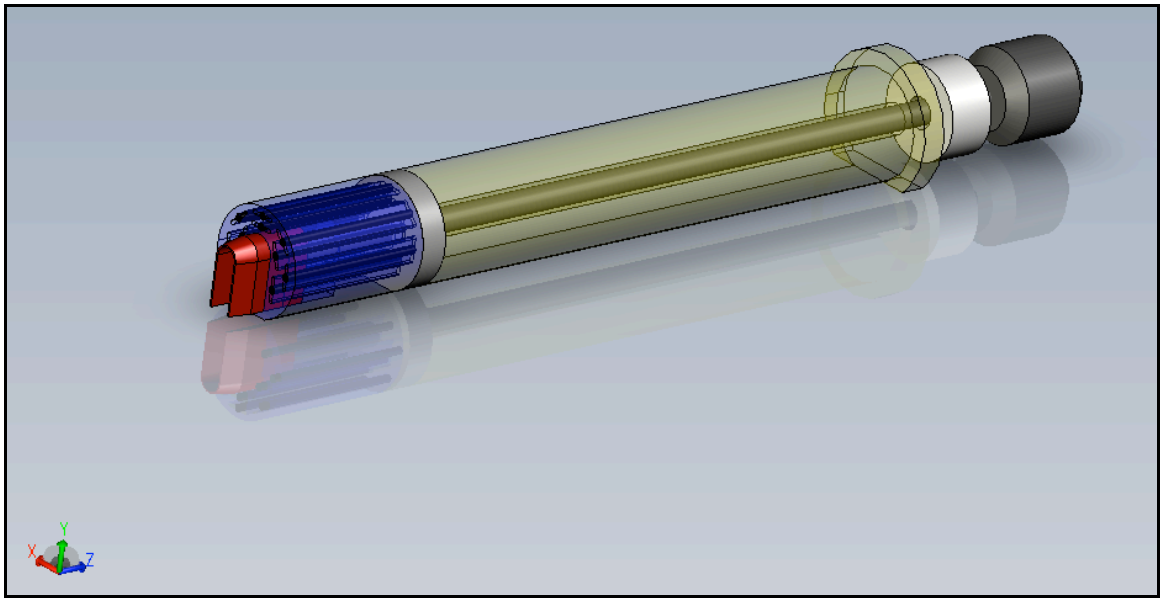


Figure 3-20 Design drawing: The inner and outer plunger and their respective components the U-shaped blade and staple cartridge. The shaft of the outer plunger is transparent and thus the shaft of the inner plunger was visualised. The image is not to scale.

Staple Design and Delivery and Staple Anvil

The intestine was loaded into the mouth of the instrument. The outer plunger was advanced so as to be in contact with the intestine. As the pressure on the outer plunger was increased the individual staple plungers advanced the staples from the staple cassettes into the intestine. The legs remained straight until they came in contact with the staple anvil and curve inwards. The base of the staple was 3mm in length; the legs of the staples were 4.8mm and the staples close to approximately 2mm. The staples were obtained from a linear stapler DST Series™ GIA™ (Ethicon Endosurgery) – a 4.8mm "single use loading unit" and thus the staples availed of directional staple technology.

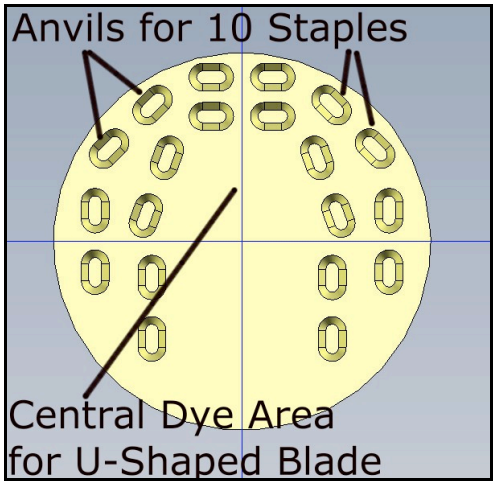


Figure 3-21 Design drawing: The dye area on the head of the Prototype Instrument 3. The U-shaped blade excises the biopsy against the central dye area. The image is not to scale.

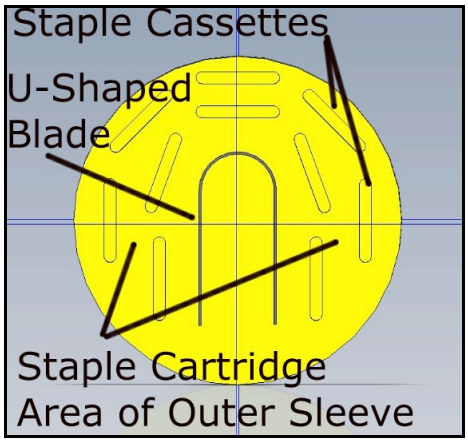


Figure 3-22 Design drawing: The U-shaped blade and staple cartridge portion of the inner and outer plunger of Prototype 3 respectively. The image is not to scale.

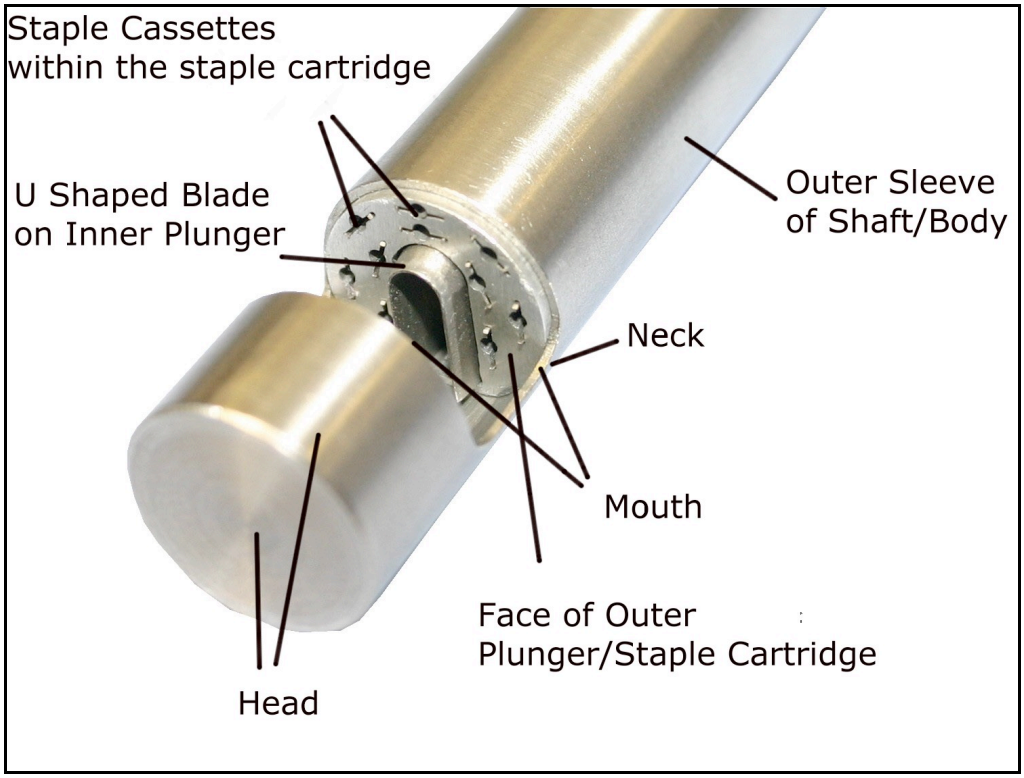


Figure 3-23 The head and mouth of Prototype Instrument 3. The image was obtained from a top to bottom angle so as to view the U-shaped blade within the staple cartridge of the outer plunger. The image is not to scale.

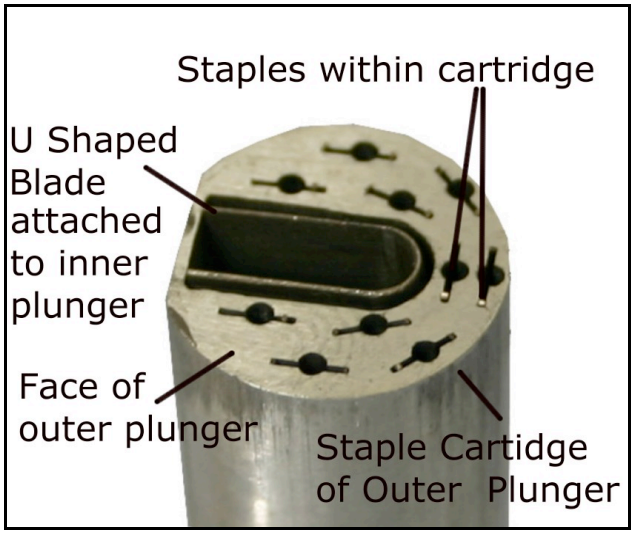


Figure 3-24 The staple cartridge of the outer plunger. The U-shaped blade is within the staple cartridge of the outer plunger. The image is not to scale.

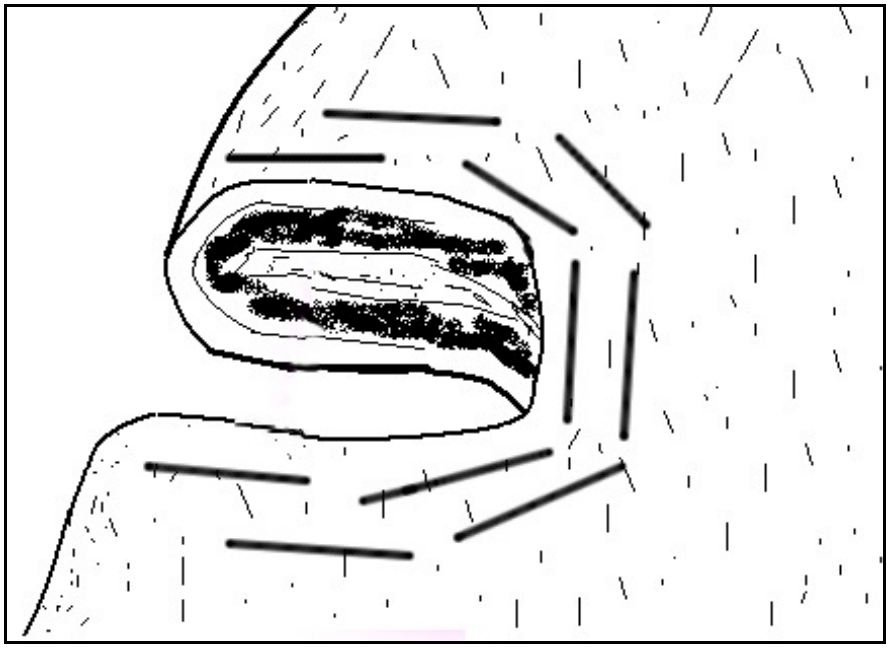


Figure 3-25 A sketch of the proposed position of the staples in a U-shaped biopsy excision closure

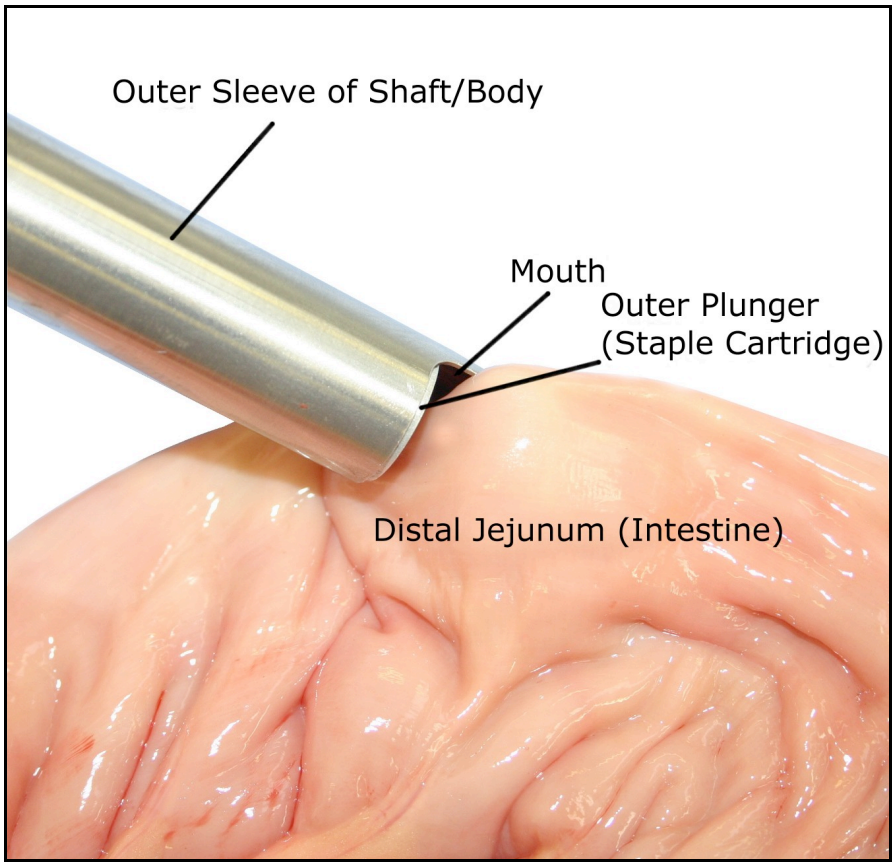


Figure 3-26 Intestine within the mouth of Prototype Instrument 3

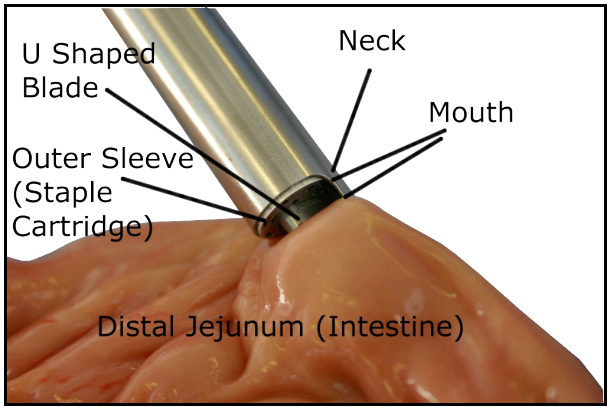


Figure 3-27 Intestine within the mouth of the Prototype Instrument 3 during biopsy excision. The inner plunger and U-shaped blade is advanced into position.

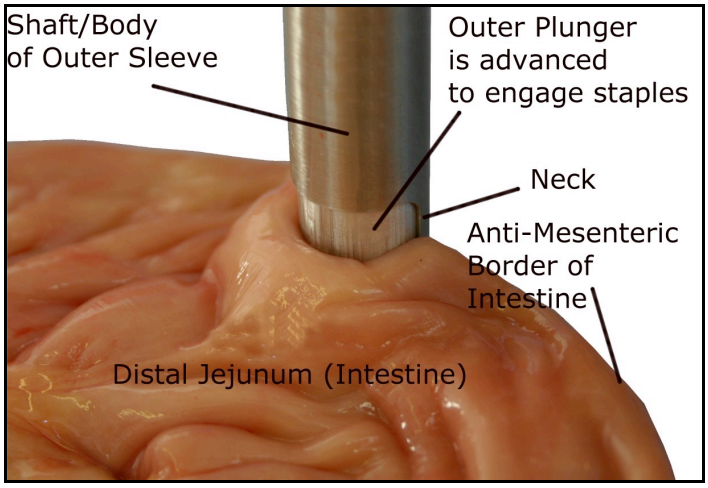


Figure 3-28 Intestine within the mouth of the Prototype Instrument 3 during biopsy excision site closure. The outer plunger has been advanced, the individual staple plungers were advanced as the intestine comes in contact with the staple cartridge.

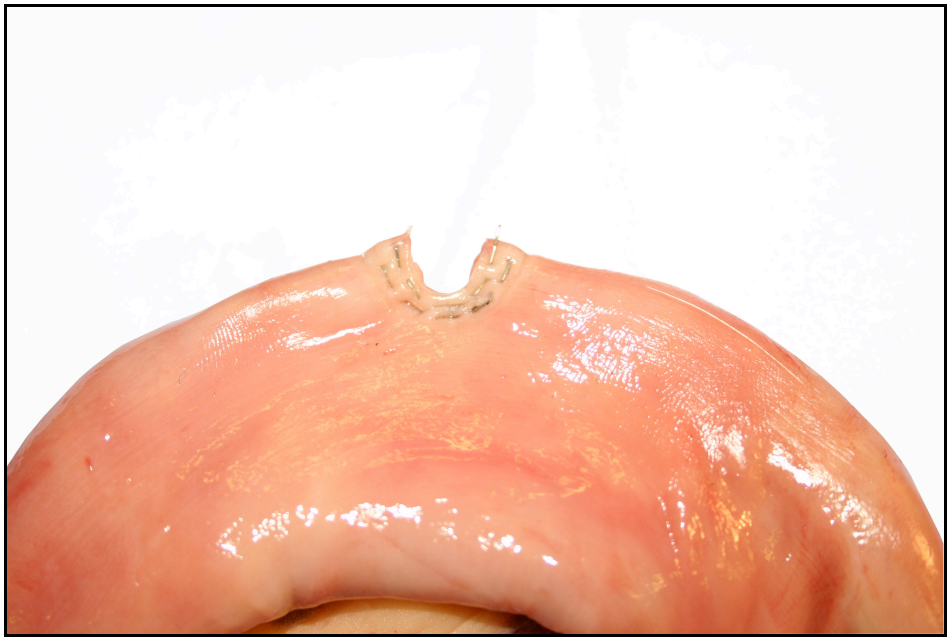


Figure 3-29 A biopsy excision site closed using a double row of staples.

Intraluminal bursting pressures

The Prototype Instrument 3 was applied between the anti-mesenteric and mesenteric border of the distal jejunum or ileum to obtain FTIB and a double row of staples as outlined previously were used to close the BES. The intraluminal bursting pressures were recorded for each of the 4 BES closures. The details are outlined in Appendix 4 and the equipment used in Appendix 6. The results obtained using Prototype Instrument 3 were compared to 4 samples of three other closure methods (outlined in Appendix 4.). The intraluminal bursting pressures are outlined in the results section using a bar graph (Figure 4-12). The percentage decrease in luminal diameter associated with each technique and the location of the bursting point are presented in Table 4-1

Chapter 4. Results

Prototype Instrument 1 – biopsy excision and gross assessment

Outer Sleeve

Positive Attributes: The size of the mouth was sufficient in this pilot study. It allowed for adequate visualisation of the intestine and was large enough to accept the intestine sufficiently for excision of the biopsy.

Required Modifications: The outer sleeve was made of copper piping. This was not of the required strength and so when the force of the screw handle was applied, it resulted in moderate bending of the neck of the outer sleeve. This resulted in the instrument being unusable. The diameter of the outer sleeve was 15mm, and the required diameter was 14.6mm to allow the instrument to fit within a 15mm laparoscopic portal. The measurements would require to be modified for future prototypes. The head of the outer sleeve was perhaps a little abrasive for manipulation within the abdomen as the ends were angular; smooth curved edges would appear to be a logical modification for a later working design to be used within the abdomen.

Detachable Blade and Plunger

Positive Attributes: The detachable blade was easily detached from the plunger and so could be replaced easily thus the blade remained sharp. The blade was securely attached. It was used for a number of excisions and worked reasonably well. The materials were readily accessible and were not expensive. The plunger was very effective and efficient.

Required Modifications: The blade was produced from a feather microtome blade. The area of the blade where the excision of the biopsy was not accurately/completely excised at the tip of the “U”. The blade bent at this point because of the force placed upon it. It was conceivable that this was the area, which was blunted by the process of bending when heated and thus why the excision was not complete at this point. Furthermore the feather blade is so called as it has a feather tip and it was believed that this was blunted after

repeated use of the blade. Perhaps another type of blade or dye would be more suitable. It was noted that the biopsy samples obtained by this method were not full thickness. On closer examination it was observed that the U-shape blade was applied to the intestine that the mucosa slipped away from the seromuscular layer. This resulted in a partial thickness sample being obtained. As in the preliminary studies it was believed that the mucosa would need to be stabilised by the use of the legs of staples or stabilised by preplaced needles. In the next prototype it was planned to introduce needle stabilisation to the detachable heads. The area around the blade was not the required 4mm in diameter and so was not sufficient to allow a double layer of staples. The position of the U-shape blade and the dimensions required modification by the engineers. It would be more economical from a space point of view to design an inner plunger to allow excision and an outer plunger to allow for ejection of staples. The outer plunger could be used in stabilisation of intestine and/or in the firing of a closure mechanism.

Handle Attachment and Screw Turn Handle

Positive Attributes: This was an efficient mechanism to power the excision of the intestine. In fact the force generated was large enough to bend the copper sleeve.

Required Modifications: The screw handle placed a lot of force on the instrument; this was positive from the point of view as it maximised the possibility of a successful excision however it may damage a future instrument even if it was a stronger construct. Furthermore, it was difficult for the operator to realise how much force was required therefore either a marker or an indicator to outline the number of rotations or the required depth would be beneficial. It was felt that the mechanism used would require a large amount of force and that spring loaded mechanisms or hand power alone would not be adequate.

Gross Assessment of Biopsy Samples

The biopsies obtained were not full thickness samples and it was apparent that the mucosa was slipping away from the serosal layers. The serosal layers were

macerated. The samples were not sent for histological assessment because the gross appearance of the biopsies were of poor quality. After a number of attempts the outer sleeve begin to noticeably bend and the prototype became unusable.

Prototype Instrument 2 - biopsy excision and histological assessment

Outer Sleeve

Positive Attributes: The walls of the outer sleeve were designed so that it would not fail under the pressure of the screw handle and could be used to obtain a number of samples.

Required Modifications: The thickness of the outer wall was most likely excessive. 2mm on either side of the outer sleeve results in a remaining approximately 10mm available for the excision and closure mechanism. It is suggested that the thickness of the outer sleeve be minimal in subsequent prototypes. However it should retain sufficient strength for it not to fail as in Prototype Instrument 1.

Screw Handle and Plunger

Positive Attributes: The screw handle delivered sufficient force for the excision of biopsies and the plunger was adequate in this prototype.

Required Modifications: The plunger was relatively cumbersome and the size was questionably too large to allow for a second plunger for the closure mechanism and should be borne in mind in the design of subsequent prototypes.

Instrument Head, Dye area and Detachable U-Shaped Blade

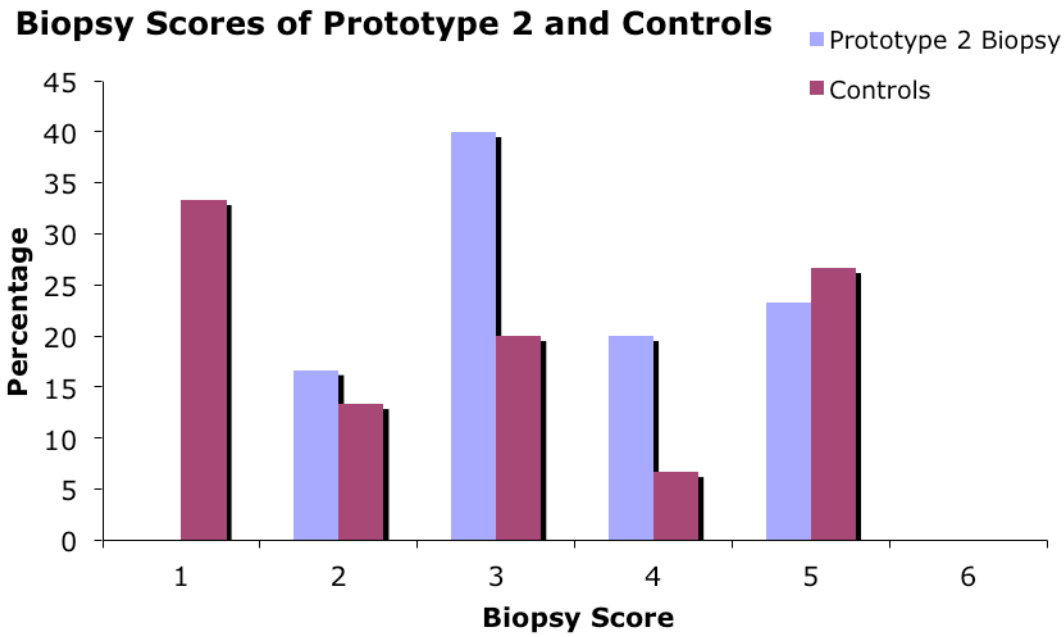
Positive Attributes: The mechanism served adequately to obtain samples for histological assessment. The needles maintained alignment and the U-Shaped Blade maintained excision capability for six samples each (however the samples became progressively more difficult to excise with each use).

Required Modifications: The portals received the advancing needle stabilisation efficiently however this resulted in difficulty in the instrument's detachable head being released easily i.e. disruption of the biopsy sample at retrieval. If the idea of the instrument having a detachable head, so that samples can be easily retrieved, is to be carried forward then there needs to be modification of this design. The dye area was flat and the samples were excised adequately for the most part. On occasion the samples were not fully excised and there were tags of serosa, which maintained a connection between the parent intestine and the biopsy sample. A suggested modification to prevent this from happening in future prototypes was to create a U-shaped groove in the dye. An alternative to the needle stabilisation was to use the legs of staples as they advance and this was used in Prototype Instrument 3.

Histological assessment

Biopsy Scores

Analysis using a 2 sample Wilcoxon rank sum (Mann-Whitney) test did not reveal a significant difference between the Prototype 2 biopsy samples (n=30) and control samples (obtained during surgery on clinical cases; n=15). Prototype 2 Biopsy and Controls both had a median grade of 3. The mean score for the Prototype 2 samples was 3.5 (standard deviation ± 1.04) and 2.8 (standard deviation ± 1.66) for the control sample. The details of the scores are outlined in Figure 4-1. The details of each biopsy are included in Appendix 5.

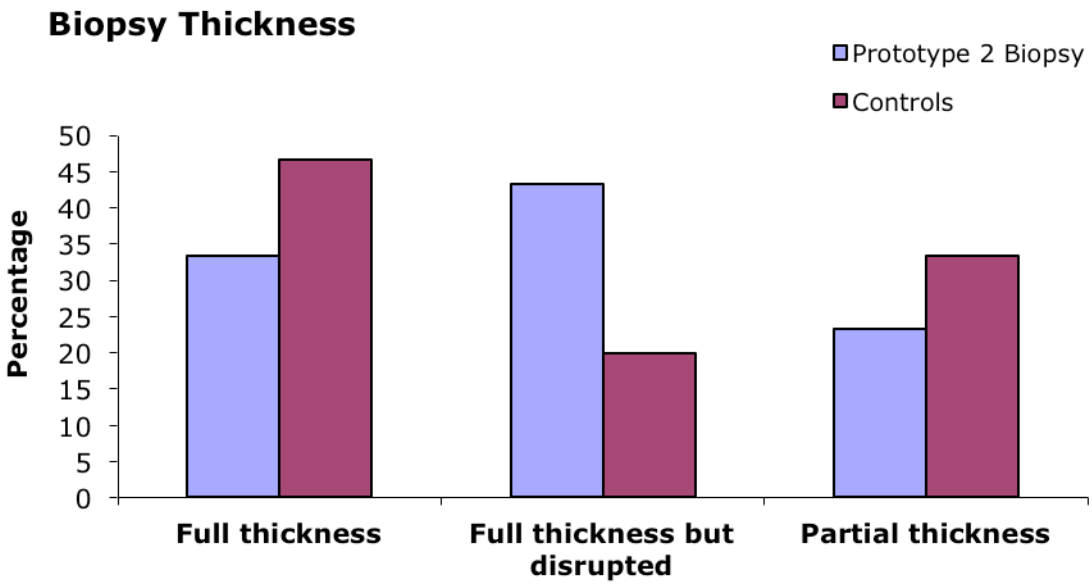


Grade	Prototype 2	Controls
1	0% (0)	33% (5)
2	17% (5)	13% (2)
3	40% (12)	20% (3)
4	20% (6)	7% (1)
5	23% (7)	27% (4)

Figure 4-1 Bar Chart: The biopsy grades of the control group (n=15) and Prototype 2 (n=30).

Biopsy Thickness

The intestinal thickness of the biopsy sampled are presented in graphical form (Figure 4-2). There were three groups - full thickness (i.e. mucosal, submucosal, muscularis and serosal layer present and not disrupted), full thickness but disrupted (i.e. all layers present as previous however there was comment on the disruption to the intestinal layers in the histological report) and partial thickness (i.e. there was a layer not presented for histological assessment – the details of the presence of each layer is presented in subsequent pages).

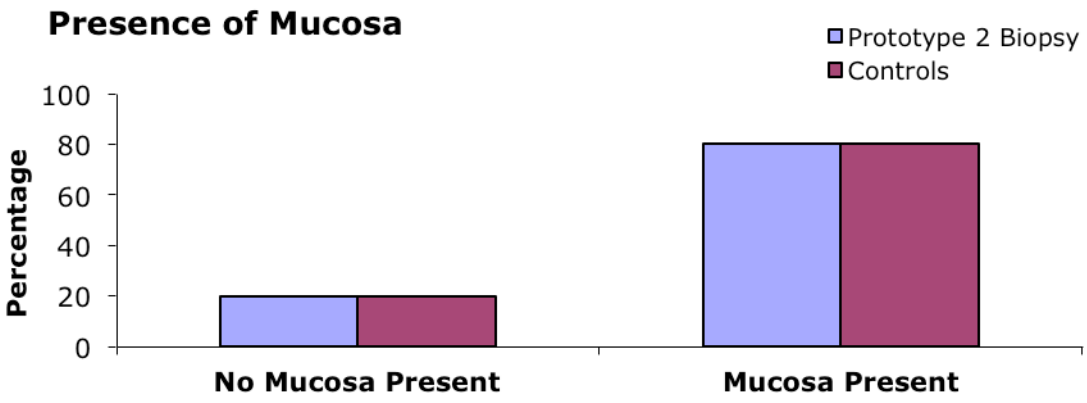


	Prototype 2	Controls
Full thickness	33% (10)	47% (7)
Full thickness but disrupted	43% (13)	20% (3)
Partial thickness	23% (7)	33% (5)

Figure 4-2 Bar Chart: Biopsy thickness for controls (n=15) and Prototype Instrument 2 (n=30).

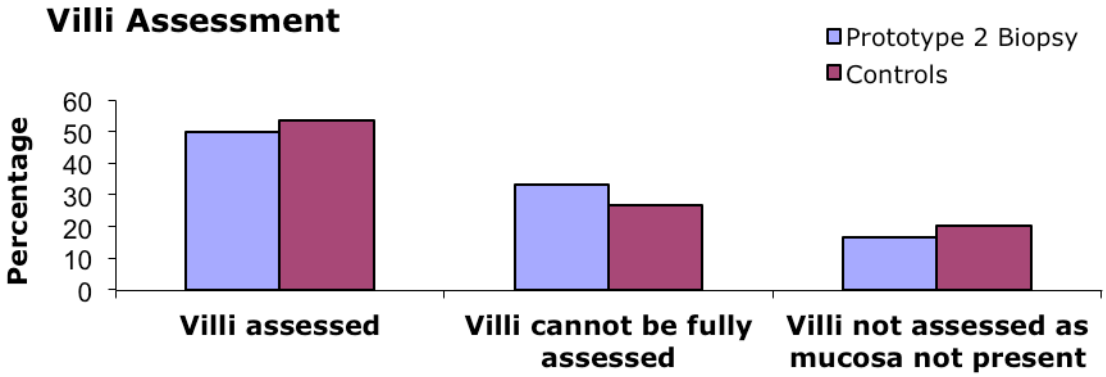
Mucosal and Submucosal Layers

The presence or absence of mucosa and the quality of the mucosa are summarised in Figure 4-3. Mucosal quality was categorised by ability to adequately assess villi. There were three groups: The first group consisted of the samples, which had adequate mucosa for assessment of quality for assessment; the second group were the biopsy samples where there was adequate sample size for assessment however the quality of the villi present was not adequate for assessment and the third group were the biopsies, that did not have adequate mucosa for assessment. The ability to assess the mucosa is presented in Figure 4-3 and the ability to assess the villi are presented in Figure 4-4. The ability to assess the submucosal layer are presented in Figure 4-5.



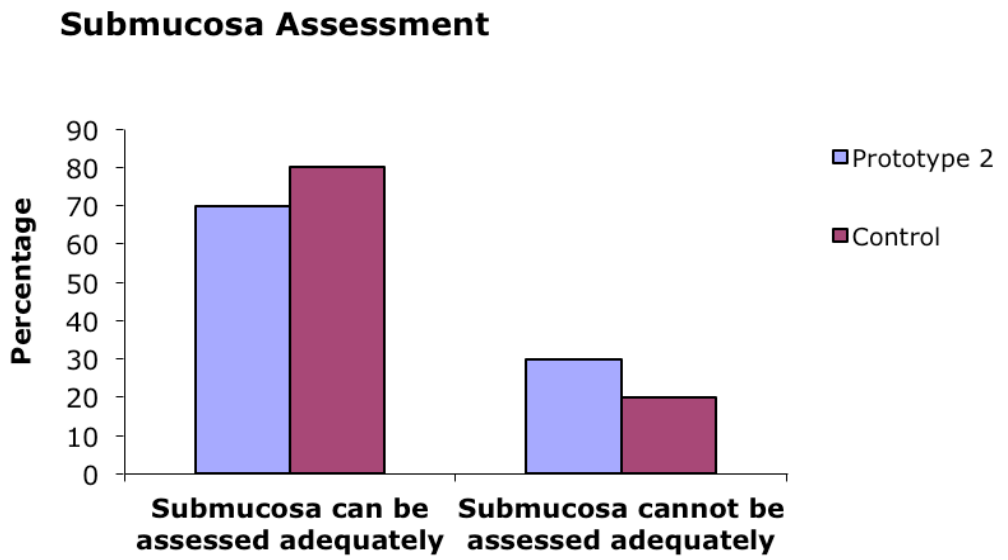
	Prototype 2	Control
No mucosa present	20% (6)	20% (3)
Mucosa present	80% (24)	80% (12)

Figure 4-3 Bar Chart: The presence of mucosa for controls (n=15) and Prototype instrument 2 (n=30).



	Prototype 2	Control
Villi assessed	50% (15)	53% (8)
Villi cannot be fully assessed	33% (10)	27% (4)
Villi not assessed as mucosa not	17% (5)	20% (3)

Figure 4-4 Bar Chart: The ability to assess the mucosal villi for controls (n=15) and Prototype instrument 2 (n=30).

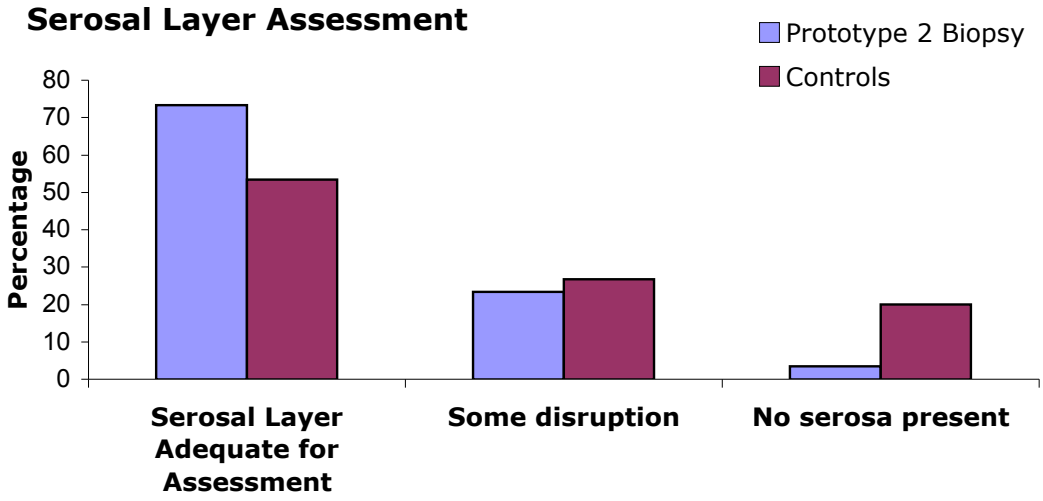


	Prototype 2	Control
Submucosa can be assessed adequately	70% (21)	80% (12)
Submucosa cannot be assessed	30% (9)	20% (3)

Figure 4-5 Bar Chart: The ability to assess the submucosal layer for controls (n=15) and Prototype Instrument 2 (n=30).

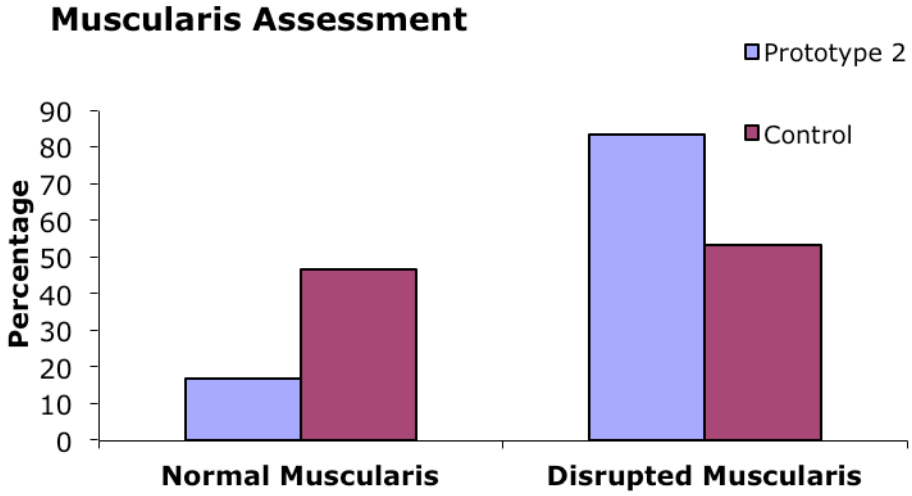
The Serosal and Muscularis Layer

The serosal layers were presented in three groups. The first group was adequate. The second group were the biopsies where there was disruption of the serosal layer histologically. The final group were the biopsies where there was no serosal layer (Figure 4-6). All the samples in both groups had a muscularis layer present for histological assessment. A number of biopsies had a degree of disruption to the muscularis layer (Figure 4-7)



	Prototype 2	Control
Serosal layer adequate for assessment	73% (22)	53% (8)
Some disruption	23% (7)	27% (4)
No serosa present	3% (1)	20% (3)

Figure 4-6 Bar Chart: The histological presence and quality of the serosal layers for controls (n=15) and Prototype Instrument 2 (n=30).



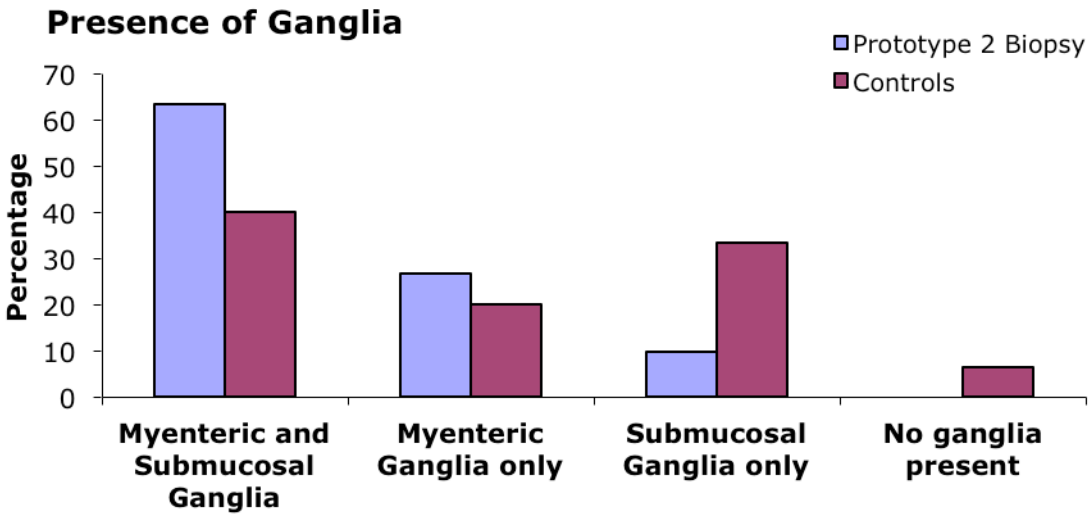
	Prototype 2	Control
Normal muscularis	17% (5)	47% (7)
Disrupted muscularis	83% (25)	53% (8)

Figure 4-7 Bar Chart: The histological presence and quality of the muscularis layers for controls (n=15) and Prototype Instrument 2 (n=30).

Myenteric and Submucosal Ganglia

The presence of ganglia was assessed histologically in all samples. The distribution of ganglia is outlined in Figure 4-8. There were four groups; the presence of ganglion in both the submucosal and myenteric ganglia, the

presence of ganglion in the submucosal ganglia or the presence of ganglion in the myenteric ganglia only and the fourth group were the biopsies without ganglion present.

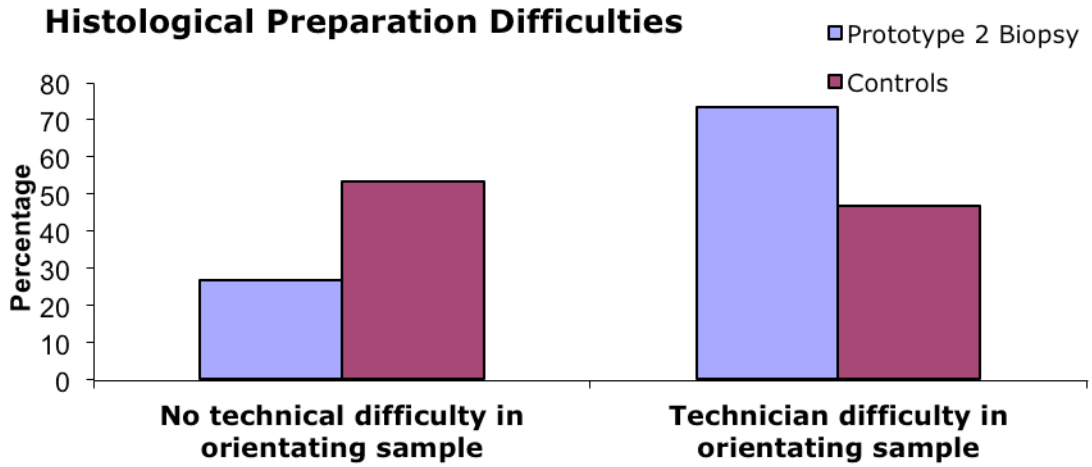


	Prototype 2	Control
Myenteric and submucosal ganglia	63% (19)	40% (6)
Myenteric ganglia only	27% (8)	20% (3)
Submucosal ganglia only	10% (3)	33% (5)
No ganglia present	0% (0)	7% (1)

Figure 4-8 Bar Chart: The histological presence of ganglia for controls (n=15) and Prototype Instrument 2 (n=30).

Histological Preparation Difficulties

As part of the histological assessment the technician was asked to record if there was difficulty in orientating and preparing the sample for histological section and the results are presented in Figure 4-9. There were two categories: the first was no difficulties in histological preparation and the second were the samples where there was some difficulty in histological preparation.

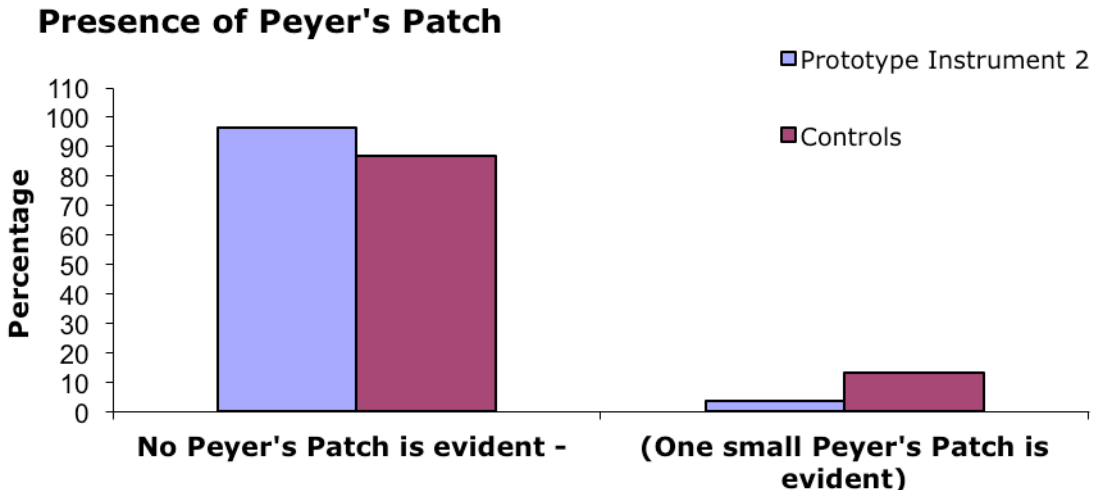


	Prototype 2	Control
No technical difficulty in orientating	27% (8)	53% (8)
Technician difficulty in orientating	73% (22)	47% (7)

Figure 4-9 Bar Chart: The difficulties in histological preparation for controls (n=15) and Prototype Instrument 2 (n=30).

The presence of Peyer's Patch

No Peyer's Patch evident histologically within the mucosa in 29/30 samples obtained from the anti-mesenteric border (Figure 4-10). In the sample where there was a Peyer's Patch it was described as follows – "A small Peyer's patch is present, consisting of several lymphoid foci with formation of a mound lined by modified epithelium ("M" cells)." There was no Peyer's Patch evident in 13 of 15 samples in the control population. In the samples where Peyer's Patch was evident it was described as follows "No true Peyer's patches were visible, but several small foci of gut-associated lymphoid tissue were present in the mucosa".



	Prototype 2	Control
No Peyer's patch is evident -	97% (29)	87% (13)
Peyer's patch evident	3% (1)	13% (2)

Figure 4-10 Bar Chart: The presence of Peyer's Patch for controls (n=15) and Prototype Instrument 2 (n=30).

Prototype Instrument 3 – closure mechanism

Outer Sleeve

Positive Attributes: The outer sleeve was thin and so the walls of the outer sleeve took up a small proportion of the diameter. The outer sleeve was robust enough to support the required actions in this experiment. Again the mouth size was appropriate for these experiments. The reinforced neck was perceived to be a useful addition in this prototype.

Required modifications: The end of the instrument was not rounded and would require modification for *in vivo* use. The length of the instrument would need to be extended so that it could be used as part of a laparoscopic instrument i.e. approximately 50cm dependent on further investigation of the optimal instrument length.

Outer Plunger, Staple Cartridge and Individual Staple Plungers

Positive attributes: The vector of action of the outer plunger was within the outer sleeve and the mechanisms were not complex. It was believed to be a relatively efficient use of the circumference of the instrument.

Required modifications: Modifications were required to ensure a more consistent and reliable closure mechanism. For example the individual staple plungers did not advance the staples consistently and evenly and thus the base of the staples deformed. The individual staple plungers rusted over time and so it became difficult to advance the outer plunger in this prototype.

Inner Plunger, U-Shaped Blade and Central Dye area

Positive attributes: The U-shaped blade appears to be stronger than the previously used U-shaped blades, which were constructed from microtome blades. The central dye area was flat. This meant that the biopsies were not always excised completely. The inner plunger was not powered by a screw handle mechanism as used in previous prototypes and so it was sometimes difficult to generate enough force to excise the biopsy fully.

Required modifications: A screw handle or similar to generate enough power so as to allow for excision. The central dye area may benefit from a U-shape grooved area or recess, which would correspond to the contact point of the blade to help ensure that the excision of the biopsy was complete.

Staple Design and Delivery and Staple Anvil

Positive Attributes: The staple ejection mechanism was a relatively simple construct. The positioning of the staples was adequate in these experiments.

Required Modifications: It was hoped to recreate the staple anvils of a linear stapler however for the staples to be arranged as illustrated the staple anvils or buckets were not the desired shape. The current staple anvils or buckets require modification to be that of a linear stapler (GIA™ –Ethicon Endosurgery). In the experiments involving prototype 3 the staples did not close in an efficient and repeatable manner. Furthermore the ends of the individual staple plungers caused deformation of the base of the staple as the contact area between the individual staple plungers and each staple was not wide enough. It would be advised that the same construct as used in a linear stapler would be employed at the end of each of the individual staple plungers to apply the appropriate and more evenly distributed pressure on the base of the staples. It is unclear from these experiments if the staple positioning is optimal. The staples at the anti-mesenteric end of the BES did not fully close on occasion as illustrated below. However, the BES remained subjectively closed when this occurred in this small sample size.

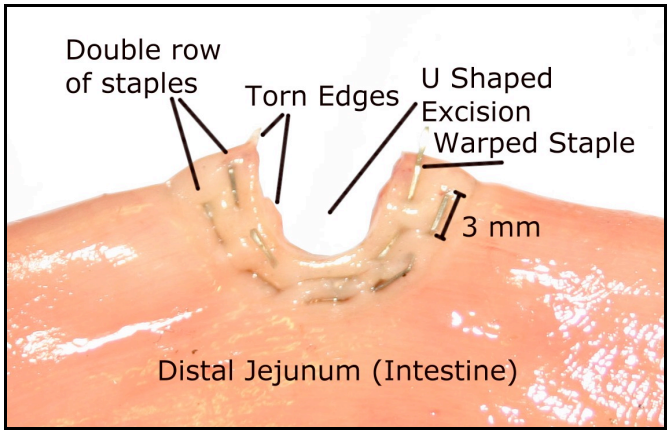


Figure 4-11 A biopsy excision site closed using a double row of staples; one of the staples was warped and the edges of the biopsy excision were frayed reflecting the poor excision technique during this biopsy excision. The image is enlarged to allow a closer examination of the biopsy excision site.

Intraluminal bursting pressures

The results of the intraluminal bursting pressure of Prototype Instrument 3 and the alternative closure techniques (Appendix4.) are outlined (Figure 4-12 and Table 4-1). The mean bursting strength were as follows: Suture Closure 295.75 mm Hg (average deviation 6.375 mm Hg), Prototype Instrument 3 185.5 mm Hg (average deviation 52.75 mm Hg), Endohernia™ 77.25 mm Hg (average deviation), Skin Staples 83.5 mm Hg (average deviation 23.5 mm Hg).

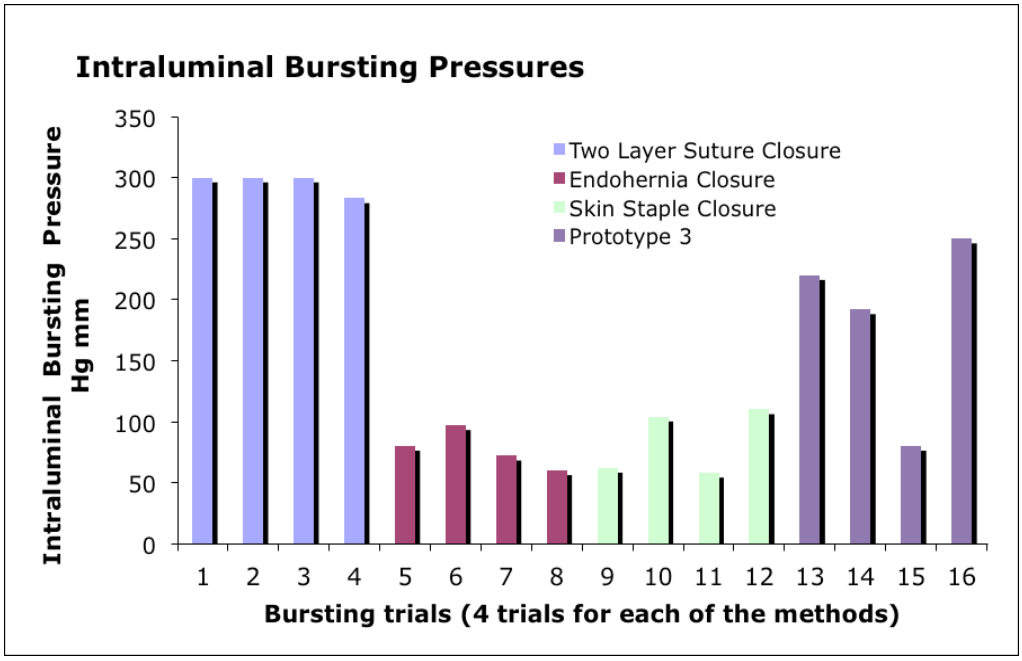


Figure 4-12 Bar Chart: Intraluminal bursting pressures of the two layer suture closure samples, the Endo hernia® closure samples, the skin staple closure samples, and of the Prototype Instrument 3 closure samples.

Closure technique	Description of Biopsy	% decrease in luminal diameter	Bursting Pressure mmHg	Bursting Point
Suture 1	U-shape from anti mesenteric border	8.6	300	Anti-mesenteric border
Suture 2	U-shape from anti mesenteric border	11.3	300	
Suture 3	U-shape from anti mesenteric border	8.9	300	
Suture 4	U-shape from anti mesenteric border	20.4	283	
Endohernia 1	U-shape from anti mesenteric border	5.2	80	Leakage at staple line
Endohernia 2	U-shape from anti mesenteric border	4	97	
Endohernia 3	U-shape from anti mesenteric border	1.8	72	
Endohernia 4	U-shape from anti mesenteric border	1.6	60	
Skin Staple 1	U-shape from anti mesenteric border	1.8	62	
Skin Staple 2	U-shape from anti mesenteric border	2.2	104	
Skin Staple 3	U-shape from anti mesenteric border	4.1	58	
Skin Staple 4	U-shape from anti mesenteric border	3.6	110	
Prototype 3	U-shape as part of Prototype 3	3.6	220	
Prototype 3	U-shape as part of Prototype 3	4.8	192	
Prototype 3	U-shape as part of Prototype 3	3.7	80	
Prototype 3	U-shape as part of Prototype 3	1.6	250	

Table 4-1 The details of each of the intra-luminal bursting pressure tests and % luminal reduction post closure of each closure method.

Chapter 5. General Discussion and Conclusion

Biopsy Excision (Prototype Instrument 1 and 2)

The preliminary investigations outlined in Appendix 4 are discussed along with the prototype instrument as this work determined the design of the prototype instrument. The most favourable techniques for the excision of a FTIB was a "cutting/punch action against the wooden bench" or a "grasping/biting" technique. These techniques were essentially similar in that there was excision against an opposing surface and the biopsy was excised in one action. The other trialled techniques in the preliminary studies were a stabbing action, scissors cutting or sawing which require a number of actions or were not against an opposing surface. It was noted that there was a large amount of force required to excise the biopsy and this was most noticeable in the grasping/biting techniques in this study. It was believed by the author that the lever to create the required forces in a grasping/biting action technique would potentially make the instrument too large. Type synthesis theory as discussed in the literature review (Lim and Erdman 2002) simplifies the relationships of the components of the instrument. The "cutting/punch action against the wooden bench" technique could be emulated along the shaft of the instrument in a linear manner thus this technique would be an ergonomically useful technique. The deformation of the copper outer sleeve in Prototype Instrument 1 demonstrated the force required to excise the biopsy.

The A5-A7 technique (Appendix 4.) could also have been obtained in a similarly linear manner. Although this technique scored well in the histological sampling the disadvantage of this technique was the inability in being able to tell if the biopsy was fully excised without damaging the apposing bowel wall. The CBT required the sample to be folded over on itself longitudinally and as a result some degree of inherent downgrading of the histological scores occurred.

The selected shape for excision was U-shaped. This shape allowed for a larger amount of mucosa to be excised as compared to a triangular shape. The rectangular shaped (A4) samples (Appendix 4.) were difficult to excise completely because either the blades were not sufficiently sharp or manufactured effectively to excise the biopsy at the corners. It was believed

that this was a manufacturing issue in the blades that were used in this set of experiments. The U-shaped blade resulted in a BES that did not have corners. This may be of benefit in the closure of the BES.

The U-shaped blade was chosen as the most useful shape and thus was chosen to determine the optimal size of a FTIB. The results of the biopsy scores suggested a trend for better biopsy scores being attributable to the 3mm width biopsy. It was possible that the 2mm biopsy would have scored similarly if the U-shaped blades were sharper and thus there would be less disruption of the sample as the biopsy was excised. Alternatively, the 3mm width of the U-shape blade could be enlarged whilst still allowing for closure of the BES. It was difficult to accurately determine the BES length as the bowel deformed as the U-shaped blade was pressed against the longitudinally folded bowel, for this reason 5-6mm was used as an approximation of the biopsy length. It was plausible that there would be a relationship between the thickness of the intestine wall and the likelihood of obtaining a FTIB i.e. a thickened bowel wall such as the most aboral ileum or pathological bowel would require a greater length of U-shaped blade to ensure a FTIB i.e. the mucosa may be excluded in thickened samples. There was some unpresented data from this study, which supports the logic of a relationship between the thickness of the intestine and the likelihood of obtaining a FTIB using the CBT. However it did not reach statistical significance in this small sample size. It was hoped that the length of 5-6mm would be sufficient for sampling jejunum and proximal ileum in the horse and should be ample for thinner intestine for example in human beings, or domestic small animals.

It was noted that stabilisation of the intestine was important in obtaining FTIB. The mucosal layers slipped away from the seromuscular layers when intestine was flattened between surfaces in both the preliminary investigations and with Prototype Instrument 1. This slippage can easily be demonstrated by placing intestine between finger and thumb and is illustrated in Appendix 6 Figure 2. The needle stabilisation method was believed to be the most useful stabilisation technique investigated in the preliminary investigations of this study. It was adapted as part of the Prototype Instrument 2. A criticism of the study is that the 4 biopsies obtained using the Prototype Instrument 3 were not examined histologically to determine if the biopsies were full thickness, the legs of the staples afforded sufficient stabilisation. It was the opinion of the author that the

gross appearance of the biopsies was in keeping with FTIB and of similar quality obtained by Prototype Instrument 2.

The biopsy samples obtained using Prototype Instrument 2 did not score statistically differently from the control population. However, it must be noted that the Prototype Instrument 2 biopsy did not score grade 1 in any of the histologically assessed samples. The major difference between the case and control samples was the disruption of the layers, which occurred in 13/30 biopsies in the case versus 3/15 in the control group. This was likely related to the Prototype Instrument 2 biopsy samples being folded and as the samples were obtained by the CBT, which inherently requires downward force on the sample.

Interestingly, the presence of mucosal layers was similar between case (24/30) (Prototype Instrument 2) and (12/15) controls. The loss of mucosal layers in the case group can be explained by the insufficient length of the biopsy samples for the BWT being sampled however this does not explain the lack of mucosal layers in the control population. Poor surgical technique, disruption of the layers between excision and histological sampling may explain why some of the control biopsies did not have histological evidence of a mucosal layer. Similarly, the case group was noticeable by the absence of serosal layers in a lower number of biopsies than the control group. Again, it was unusual and unexplained that the FTIB obtained by conventional means did not have serosa present in 3/15 cases (20% of samples).

The Prototype Instrument 2 biopsy samples were inherently folded samples and it was not surprising that the technician recorded some difficulty in orientating the samples in 22/30 cases. However, the technical difficulty was not to a level, which resulted in a statistical difference in the biopsy grades. It also must be remembered that the samples obtained by the Prototype Instrument 2 were presumed to be smaller than that obtained conventionally. For example Mair *et al*. (2002) describes a full thickness ellipse of the ileum 3 x 1cm in size to be obtained for histological examination of a FTIB. This amounts to an area of $3cm^2$ whilst the approximate area of a Prototype Instrument 2 U-shaped excision would be $0.36cm^2$. The latter mentioned approximation was calculated by the following: 6mm in length by 3mm in width by 2 (i.e. both sides of the folded piece of intestine). This approximation does not account for many possible

variations such as the BWT, the stretching of intestine when the CBT causes deformation of the sample and the shrinkage of the samples due to fixation among other possible variations. However, it was our clinical impression that the biopsy samples were smaller than that conventionally obtained. There were a number of possible positive consequences as a result of a smaller biopsy size such as quicker healing of the BES and earlier return of the intestine to normal function and potentially less chance of wound dehiscence and resultant abdominal contamination.

No true Peyer's patches were present during the histological examination, but several small foci of gut-associated lymphoid tissue were present in the mucosa of the control group. These samples were obtained in the standard fashion midway between the mesenteric and anti-mesenteric border of the small intestine. There was one small Peyer's patch visible in the case group obtained by Prototype Instrument 2. The size of the Peyer's patch did not prevent histological assessment of the sample in this case. These samples were obtained from the anti-mesenteric border of the intestine. It has been described that small intestinal biopsies should be obtained from midway between the mesenteric and anti-mesenteric border of the small intestine (Mair 2002). The reasoning for this was to avoid the Peyer's patch. It was interesting that all of the samples obtained from the anti-mesenteric border in this study were capable of histological assessment.

No attempt was made to draw a statistical conclusion between the groups except for the biopsy score as there was a small sample size in both the case and control groups i.e. 30 and 15 subjects respectively.

Further investigation would be required to ensure that the mechanism for the excision of the biopsy was complete as, on occasion, the blade sharpness and/or downward pressure was not sufficient to excise the biopsy completely. Perhaps the addition of a U-shaped dye to fit the U-shaped blade measuring for example 1 mm in depth as apposed to a flat dye present in the current format would be of benefit. Alternatively a single use blade could be used. This would have the advantage of being sharp and could be designed to detach and contain the biopsy within. The screw handle plunger similar to the design in Prototype Instrument 2 or a variation of this design resulting in similar pressure (Similar to

that used in a TA-90®, Covidien) should be adopted in future prototypes as the downward pressure to achieve a FTIB was greater than that achieved comfortably by a simple manual technique as employed by the inner plunger of Prototype Instrument 3. Further investigation would need to address the ergonomics of laparoscopic instruments and the most suitable handle and firing mechanisms both in the excision and actuation of the excision and closure mechanism.

Biopsy Excision Site Closure (Prototype Instrument 3)

There was a long list of possible closure methods available to close the U-shaped BES. The secondary closure methods such as skin staples, and the Endohernia™ and the suture method require a second instrument within the abdomen or the potential modification/adaption of the excision instrument to include the closure method. The results of the intraluminal bursting pressures revealed that the skin staples and the Endohernia™ closure method were associated with lower pressures than the Prototype Instrument 3 closure method. It was apparent that once the samples were excised the subsequent delay in closure of the BES resulted in time for the BES to deform thus making the placement of staples such as these difficult to achieve. In a clinical case it would likely be that there may be contamination of the abdomen using a secondary closure technique. A primary closure technique would be advantageous as outlined in Prototype Instrument 3.

The mean intra-luminal bursting pressure was greater for the Prototype Instrument 3 in comparison to the Endohernia™ and Skin Staples. It was the opinion of the author that the latter were not satisfactory closure methods and that the Prototype Instrument 3 closure method was not as a robust a closure method as suture closure. Although it was not possible to draw statistical conclusions from the small number of samples tested, the mean intra-luminal bursting pressure supported this idea. Interestingly, the suture closed samples had bursting sites away from the BES (Table 4-1). Furthermore, the experimental setup to test the intra-luminal bursting pressure, although sufficient for assessment of a small number of closure methods, would benefit from sophistication in a larger study where more rigorous statistical analysis would be possible. The luminal diameter was reduced greatest by the double layer of sutures. The Prototype 3 instrument compared favourably with the sutured samples (Table 4-1). The results suggested that the luminal reduction was greatest when using the suture closure (Table 4-1). It is possible that the double layer of continuous Cushing's suture may have exaggerated the luminal reduction that is normally achieved in clinical cases.

Resources, time and money limited the scope of the investigation of a biopsy closure method. To the author's knowledge modification of a linear row of

staples to the shape outlined in Prototype Instrument 3 was a new concept. The positioning of the staples was adequate in this assessment. The staple mechanism was not as proficient as would be required in a production model in live patients. A number of staple ejections failed and a revision of the closure mechanism whilst maintaining the double row of staples within the same staple cartridge and individual staple plungers would be required as the actuation of the staple mechanism was not reliable in its current format. The closure of the BES results in the apposition of the intestinal layers i.e. an everted closure. This results in the potential contamination of the abdomen. This closure was similar to the use of a linear staple to transect intestine such as used by Mazziotti (2001), Greig et al. (1995) and Schambourg and Marcoux (2006) and was similar to the use of a linear stapler resulting in an everted closure by a number of other techniques widely in use both in human and veterinary medicine (Tobias 2007). Linear staplers are often used to form an inverting pattern as employed in the technique described for jejunocaecal anastomosis as described by Freeman (1997) among many other similar techniques. This prototype instrument has the potential to be used in a similar manner. For example, if the instrument was introduced intra-luminally and a fold of intestine was introduced into the mouth of Prototype instrument 3 so that a full thickness sample would be retrieved in a similar but reverse fashion than that achieved extra-luminally. The current extra-luminal technique forms a folded FTIB sample, which results in the inner layer being mucosa and the outer curvature of the sample being serosa. An intra-luminally excised biopsy would result in the serosa being on the inner curvature of the folded sample and the mucosa on the outer curvature. The introduction of the instrument intra-luminally could be achieved per rectum or as per a Natural Orifice Translumenal Endoscopic Surgery or modified laparoscopic procedure. A theoretical usefulness of this technique would be the attainment of FTIB via the rectum with an inverted BES closure; the location of the biopsy could be dorsal to avail of the protective mesenteric attachments to limit potential contamination. Wales and Whitwell (2006) described the use of full thickness biopsy of the rectum in post mortem cases to diagnose Grass Sickness in horses. This idea was a theoretical and potential benefit of this instrument design.

Conclusion

This thesis outlines the investigation of a laparoscopic FTIB instrument. The investigation was accomplished by the identification of the requirements of a FTIB and subsequently identification of the limiting steps i.e. excision and closure of the BES. A scoring system was designed based on the requirements. The chosen excision and closure techniques were adopted into prototype instruments. The step-wise decision making investigative process could be adopted for use in developing other laparoscopic and surgical instrumentation.

A novel biopsy excision method was investigated and results have shown the biopsy samples to be comparable to a control population. A prototype closure mechanism was also identified and was shown to be a potentially useful closure mechanism as per intraluminal bursting pressures in an experimental setup. The U-shaped blade mechanism using the CBT with needle or staple stabilisation has the potential to be adapted to a number of minimally invasive procedures to obtain extraluminal or intraluminal biopsy samples.

Appendix 1. List of manufacturers

BD, 1 Becton Drive, Franklin Lakes, NJ USA 07417

Covidien plc., 20 Lower Hatch Street, Dublin 2, Ireland

CP Lab Safety - 14 Commercial Blvd Suite 113 - Novato, CA 94949

Ethicon Endosurgery, 4545 Creek Road, Cincinnati, OH 45242, United States

Kontron Medical SAS, Zone d'activite des Gatines, 52 rue Pierre Curie, Plaisir, F-78373, France

L. Robinson & Company (Gillingham) Limited Owens Way, Gads Hill, Gillingham, Kent, ME7 2RS, UK

Surgipath Europe Ltd., Venture Park, Stirling Way Bretton, Peterborough, PE3 8YD, UK

Solidworks, Waltham, Massachusetts, USA

Swann-Morton Ltd., Owlerton, Sheffield, S6 2BJ, UK

Appendix 2. Details of materials and methods

Study Part	Horse	Intestinal Section	Time from euthanasia	Details	Reason for Euthanasia
Screening 1-5	A	Ileum and distal jejunum	Approx. three hours	8 year old Bay Thoroughbred Gelding	Unrelated to abdominal disease
Screening of A1-A8	B	Ileum and distal jejunum	Approx. three hours	10 year old Grey, Irish Draft Cross, gelding	Euthanasia related to on going lameness
Screening of A1-A8	C	Ileum and distal Jejunum	Three hours	5 year old Bay Cob Gelding	Unrelated to abdominal disease
Screening of Shapes	D	Ileum and distal Jejunum	6-12 hours	5 year old Thoroughbred Cross Gelding	Unrelated to abdominal disease
Screening of Sizes	E	Ileum and distal Jejunum	6-12 hours	14 year old Connemara Cross Mare	Unrelated to abdominal disease
Screening of Sizes	F	Ileum and distal Jejunum	6-12 hours	8 year old Highland Pony Cob Mare	Unrelated to abdominal disease
Screening of Closure 1-3	G	Ileum and Jejunum	6-12 hours	Aged >15 year old Thoroughbred, Gelding	Unrelated to abdominal disease
Screening of Closure 1-3	H	Ileum and Jejunum	6-12 hours	14 year old Dutch Warmblood Gelding	Unrelated to abdominal disease
Screening of Closure 1-3	I	Ileum and Jejunum	6-12 hours	16 year old Welsh Section A Pony Mare	Unrelated to abdominal disease
Prototype Trial 1	J	Ileum and distal Jejunum	Less than 3 hours	3 year old Thoroughbred Colt	Unrelated to abdominal disease
Prototype	K	Ileum and	Less than 3 hours	2 year old Thoroughbred	Unrelated to

Trial 2		distal Jejunum		Colt	abdominal disease
Prototype Trial 2	L	Ileum and distal Jejunum	Less than 3 hours	8 year old Thoroughbred Mare	Unrelated to abdominal disease
Prototype Trial 2	M	Ileum and distal Jejunum	Less than 3 hours	20 year old Irish Sport Horse Gelding	Unrelated to abdominal disease
Prototype Trial 2	N	Ileum and distal Jejunum	Less than 3 hours	18 year old Welsh Section D Cob Gelding	Unrelated to abdominal disease
Prototype Trial 2	O	Ileum and distal Jejunum	Less than 3 hours	4 year old Cob Pony Mare	Unrelated to abdominal disease
Prototype Trial 3 Closure	P	Ileum and Jejunum	Less than 3 hours	3 year old Thoroughbred Gelding	Comminuted carpal fracture
Prototype Trial 3 Closure	Q	Ileum and Jejunum	Less than 3 hours	4 year old Thoroughbred Gelding	Catastrophic Fracture of the tibia

Appendix 2 Table 1 The table outlines the signalment of the different horses used in the different parts of the study and the timing and cause of death.

Appendix 3. Preliminary Investigations 1: Requirements, Evaluation Criteria, Scoring System and Histological Assessment

Introduction

There are a number of important requirements of any potential laparoscopic instrument. The functional requirements of a laparoscopic FTIB instrument are the necessary task, action or activity that must be accomplished i.e. the excision of the biopsy, the closure of the BES. There were also other essential and non-essential constraints and capabilities. For example a constraint on the project was the budget allotted and so expensive materials would not be possible in the development of prototypes and that the number of engineers involved and their time would be limited to what could be afforded. Examples of capabilities were the ability to fit within a cannula, be easy to use and be reliable. These requirements are used as evaluation criteria during decision-making in the design process. Essentially without excision of the biopsy there cannot be a biopsy instrument and this was the first area investigated. The second limitation is the closure of the BES.

A process is a logical sequence of tasks performed to achieve a particular objective. It defines "What" is to be done without specifying "How" each task is to be performed. The structure of a process provides several levels of aggregation to allow analysis and definition to be done at various levels of detail. A process phase consists of tasks/methods, and a task may consist of several steps, decompositions and levels of aggregation (Martin 1997). The requirements of the instrument were listed. The requirements overlapped and so these were re-categorised as evaluation criteria and adopted as part of a scoring system as outlined under the headings "Requirements of a laparoscopic full thickness biopsy instrument", "Evaluation Criteria" and "Scoring system to assess biopsy excision and biopsy closure techniques". Firstly, the possible tasks are identified for biopsy excision and closure (Appendix 4 "Identification of possible tasks"). Secondly, the task is screened (Appendix 4 "Screening"). Finally, the most appropriate tasks were chosen to carry out the process of biopsy excision and biopsy closure and these were introduced into Prototype Instrument as outlined in the main body of the thesis. Evaluation of the biopsy

excision was by a histological assessment (outlined in Appendix 3. Histological Assessment) and was used to evaluate the biopsy quality both in the preliminary investigations (Appendix 4.) and Prototype Instrument 2 (Chapter 3 and 4). Intraluminal bursting pressures were used to evaluate the biopsy excision site closure techniques (Appendix 4.) and Prototype Instrument 3 (Chapter 3 and 4).

Requirements of a laparoscopic full thickness biopsy instrument

There are a number of important requirements of any potential laparoscopic instrument and these relate to patient requirements, surgeon's requirements and production and maintenance requirements. A literature investigation of intestinal biopsy was carried out and the following requirements were identified.

Patient related requirements

Minimal amount of tissue handling, and sensitive tissue handling

Rapid completion of surgery

Ideally the instrument should be capable of obtaining biopsy from all segments of intestine (i.e. jejunum, duodenum, ileum, the ascending and descending colon.) and should be capable of a biopsy through multiple wall thicknesses

No contamination of abdomen during or post surgery

Early return of intestine to normal function

The biopsy excision should be small so as to minimise the chance of luminal stenosis

Small sized laparoscopic portals and as few as possible (ideally one for camera, one for biopsy instrument) to minimise physiological effects of a surgical incision

dimensions should be so as to fit within a maximum laparoscopic portal diameter of 15mm, the total instrument dimensions in a transverse plane should be limited to 14mm or less

Surgeon related requirements

One step procedure with easy to use functions

Stabilisation and selection of the intestine should be done with the biopsy instrument and the retrieval of the biopsy should not involve another instrument

The instrument should allow visualisation of the biopsy site

The quality of the biopsy from a histopathological aspect should be excellent

Production and maintenance requirements

Should be reusable within an operation to allow multiple biopsy sites (this may require reloading of a ligature/stapling device)

Economic to produce

Be of appropriate material

Evaluation criteria

There was much over lapping of the requirements and thus a simplification was carried out resulting in a list of Evaluation Criteria.

Gross assessment of biopsy sample

The gold standard in biopsy assessment is by histologically however it is useful in herein to grossly assess the biopsy. It is assumed that the biopsy sample should be of adequate size (see Biopsy Size), include all of the intestinal layers and should be consistently well formed with minimal disruption of the sample and be repeatable between samples.

Biopsy excision site

A biopsy excision, which is well formed and delineated and repeatable, is presumed to be easier and more likely to be successfully closed than a disorganised, variable excision site resulting in no contamination of the abdomen. An excision that is large, of variable size and consistency (ragged or torn edges) is perceived to be more likely to result in contamination of the abdomen. Furthermore the biopsy closure method must prevent contamination both in the short term and facilitate healing so a quick return to normal intestinal function is possible.

Biopsy size

There is a balance between biopsy size and histological quality. Furthermore the smaller the biopsy is, the less intestinal trauma, less chance of luminal stenosis, and quicker return to normal function. However, it is important to maintain a well-formed sample that would be likely to be of an adequate standard for histological examination. A detailed histological examination of different biopsy size was carried out separately in a later section of the investigation. The scores outlined in Appendix 3 were used to estimate the prospective biopsy size of a technique whilst maintaining a well-formed sample that was likely to be of an adequate standard for histological examination. A detailed histological examination of different biopsy size is carried out in a later section of the investigation. The measurements were devised based on a theoretical minimal sized biopsy and the sizes of biopsy as per reported in the literature (Scholes *et al*. 1993a, Mair 2002). 1.5cm^2 is the recommended size of biopsy using the standard excision techniques to obtain ileal biopsy (Scholes *et al*. 1993a). The minimal size of the histological section to be useful for histological assessment was suggested to be 3mm in length by the depth of the intestine (i.e. the distance between the serosal layer and the inner most aspect of the mucosa) by the thickness of the section which in the case of a histological section is 10 μm (Van der Lubbe *et al*. 1988). Tissue contraction of the histological sections can occur after fixation in formalin (Margo and Lee 1995) as a result of this phenomenon a safety factor of 25% was estimated which resulted in an optimal biopsy of 3.75mm x 3.75mm by the depth or thickness of the intestinal layers. However it must be remembered that any optimal size is theoretical and

individual to this investigation as it was largely influenced by the constraints and requirements of this project.

Intestinal thickness versatility

Ideally a biopsy instrument would be capable of excision through different sections of the intestinal tract and through different wall thickness of biopsy. Only the distal jejunum and ileum were used in this study. There was a notable difference in the thickness of the small intestine between the thick walled muscular ileum and the thinner distal jejunum. The use of this section of the intestine was selected as it is suitable for the diagnosis of Grass Sickness however it was also useful as the bowel wall thickness (BWT) is variable from the distal jejunum to the distal ileum and thus allows for the instrument to be trailed on a variety of intestinal thickness.

Luminal stenosis

It would be logical to presume that the biopsy size and shape would be related to the defect or size of the BES and thus the amount of tissue required to close the defect would be one of the factors likely to contribute to the degree of luminal stenosis. Furthermore, the closure method would influence the degree of stenosis caused by the amount of tissue involved and the type of apposition of the intestine. If the intestinal walls were perfectly apposed then there would be little luminal stenosis as compared to a closure method involving eversion or inversion or where the intestine was closed on itself in a sandwich-like closure, which would include more intestinal tissue or the luminal diameter and lead to a greater degree of luminal stenosis.

Task complexity

Ideally the methods will result in a one step procedure that will allow for excision and closure of the biopsy simultaneously thus limiting contamination, surgery time and tissue handling and also have easy to use functions. It would be logical to presume that the fewer steps and less technical skill required the more easy to use the functions would be and manufacture. The scoring system outlined details the perceived complexity of each technique as regards the

number of actions required and the number of instruments required in the excision and closure of the FTIB.

Instrument size

The instrument will require to be introduced to the abdomen via 15mm cannula and the different tasks of the processes of excision and closure to act within the confines of the abdomen. Furthermore, as stated previously, one of the aims is to design a simple and easy to use instrument and thus it is most likely that the force of the actions should be generated by a handle mechanism extracorporeally yet the action will be carried out intracorporeally. Additionally it is perceived to be advantageous if the vectors associated with the required actions were parallel to the shaft handle as this would result in simplification of the mechanisms involved in the desired actions and thus result in a less cumbersome instrument. The scoring system is based on the perceived ability of the techniques and instruments to operate via a cannula.

Stabilisation and selection of the intestine

Ideally the instrument should be capable of selection and stabilisation of the tissue and thus decrease surgery time and tissue handling. Each technique is scored as regards the perceived complexity of the stabilisation and selection of the intestine. Furthermore if the technique requires a lot of or robust tissue handling it is assumed to be detrimental to the patient.

Scoring system to assess biopsy excision and biopsy closure techniques

Gross assessment of biopsy sample

1= the biopsies are repeatably (90 - 100% of samples) well formed with minimal disruption to the intestinal layers and consistently symmetrical in shape and the biopsy sample are not folded and include all of the intestinal layers.

2= the biopsies are consistently (70% - 90% of samples) well formed with minimal disruption to the intestinal layers and symmetrical in shape and the biopsy samples include all of the intestinal layers; the biopsy quality was repeatable

(70 - 90% or greater of samples are of similar quality and when the quality varies it was a minor variation for example incomplete excision of all intestinal layers, minor technical failure etc.).

3= the biopsies are consistently (70 - 90% of samples) well formed with minimal disruption to the intestinal layers; the biopsy sample include all of the intestinal layers however the shape and symmetry may vary; the biopsy quality was repeatable (70% or greater of samples should be of similar quality and when the quality varies it should be a minor variation for example incomplete excision, minor technical failure etc.).

4= the biopsies are often not well formed (less than 70%) and there was often disruption to the intestinal layers; the biopsy sample often include all of the intestinal layers however the shape and symmetry vary often (less than 70% of biopsy samples are similar).

5= the biopsies are often not well formed and often do not contain all of the intestinal layers

Biopsy Excision Site

1= All the biopsy samples are fully and successfully excised and in all samples the intestinal walls of the BES and the layers of the intestine are aligned and well formed in a symmetrical fashion between the different parts of the BES.

2= Most of the biopsy samples are fully and successfully excised and retrieved or on occasion a small fibrous-like tag from an intestinal layer remains which was easily removed once the biopsy sample was removed. In addition all samples the intestinal walls of the BES and the layers of the intestine are aligned and well formed in a symmetrical fashion between the different parts of the BES.

3= Most of the biopsy samples are fully and successfully excised and retrieved however the intestinal walls are not consistently aligned and well formed in a symmetrical fashion between the different parts of the BES. When the BES does not fulfil these criteria the BES intestinal walls are moderately disrupted

resulting in a notable separation of the intestinal layers and/or a variability in the serosal excision.

4= Approximately half of the biopsy samples are fully and successfully excised and retrieved however the intestinal walls are rarely aligned and well formed in a symmetrical fashion between the different parts of the BES. When the BES are not well aligned and well formed the BES intestinal walls are markedly disrupted resulting in a marked separation of the intestinal layers and/or a variability in the serosal excision.

5= A number of attempts are required to fully excise and retrieve the biopsy resulting in asymmetrical, misaligned and poorly formed intestinal walls and layers of the BES.

Biopsy size

1= The achieved biopsy size or it was conceivable that the technique would result in a biopsy size less than or equal to 0.14cm^2

2= The achieved biopsy size or it was conceivable that the technique would result in a biopsy size between 0.14cm^2 and 0.5cm^2

3= The achieved biopsy size or it was conceivable that the technique would result in a biopsy size between 0.5cm^2 and 1.5cm^2

4= The achieved biopsy size or it was conceivable that the technique would result in a biopsy size between 1.5cm^2 and 3cm^2

5= The achieved biopsy size or it was conceivable that the technique would result in a biopsy size greater than 3cm^2

Intestinal thickness versatility

1= Capable of or it was conceivable that the technique would result in excision and/or closure of biopsy samples from all intestinal thickness and in achieving a sufficient excision of all of the layers of the intestine (most notably the mucosa)

2= Capable of or it was conceivable that the technique would result in excision and/or closure of most biopsy samples but not full thickness samples from intestine which was excessively thickened i.e. in intestine with greater than 2cm BWT

3= Capable of or it was conceivable that the technique would result in excision and/or closure of most biopsy samples of normal intestine but not intestine of a bowel wall thickness of greater than 0.7cm BWT

4= Capable of or it was conceivable that the technique would result in excision and/or closure of most biopsy samples from normal or thin samples i.e. intestine with greater than 0.4cm BWT

5= Rarely or unlikely to be capable of excision and/or closure of biopsy samples from any intestine location and thicknesses

Luminal Stenosis

1= A small BES and a shape which would result in a minimally sized mucosal defect and the BES would be likely to be closed with minimal narrowing of the luminal diameter

2= A small biopsy BES and a shape which would result in a minimally sized mucosal defect and the BES would be likely to be closed with an acceptable amount of luminal diameter narrowing

3= A medium sized BES and a shape which would result in a medium sized mucosal defect and the BES would be likely to be closed with an acceptable amount of luminal diameter narrowing

4= A medium sized BES and a shape which would result in a medium sized mucosal defect and the BES would be likely to be closed with an unacceptable amount of luminal diameter narrowing

5= A large sized BES and a shape which would result in a large sized mucosal defect and the BES would be likely to be closed with an unacceptable amount of luminal diameter narrowing

Task complexity

1= Requiring or it was conceivable that the method would require one instrument and one action required to excise FTIB and close the BES

2= Requiring or it was conceivable that the method would require one instrument and two actions required to excise FTIB and close the BES

3= Requiring or it was conceivable that the method would require one instrument and three or four actions required to excise the FTIB and close the BES or two instruments and two separate actions required to excise the FTIB and close the BES

4= Requiring or it was conceivable that the method would require one instrument requiring greater than 4 actions required to excise the FTIB and close the BES or two instruments and a number of actions required to excise the FTIB and close the BES

5= Requiring or it was conceivable that the method would require multiple instruments and multiple actions required to excise the FTIB and close the BES

Instrument size

1= it was planned that the circumference of the instrument will fit within a 6mm or less in diameter cannula and the vectors associated with the action(s) of excision of the FTIB and closure of the BES are parallel to the shaft handle.

2= it was planned the circumference of the instrument fits within a 12mm or less in diameter cannula and the vectors associated with the action(s) of excision of the FTIB and closure of the BES are parallel to the shaft handle.

3= it was planned the circumference of the instrument fits within a 15mm or less in diameter cannula and the vectors associated with the action(s) of excision of the FTIB and closure of the BES are parallel to the shaft handle.

4=it was planned the circumference of the instrument fits within a greater than 15mm in diameter cannula and the vectors associated with the action(s) of excision of the FTIB and closure of the BES are parallel to the shaft handle.

5= it was planned the circumference of the instrument fits within a greater than 15mm in diameter cannula and the vectors associated with the action(s) of excision of the FTIB and closure of the BES are not parallel to the shaft handle. This requires a number of driving mechanisms which may result in a larger size instrument most especially if it was attempted to be incorporated in a one step and one instrument technique.

Stabilisation and selection of the intestine

1= the technique was planned to require no intestinal selection or stabilisation and it was believed that the FTIB could be obtained and the BES closed without the intestine being held by another instrument.

2= the technique was planned to require minimal intestinal selection and stabilisation and it was believed that the FTIB could be obtained and the BES closed with the aid of a simple modification of the same instrument.

3= the technique was planned to require minimal intestinal selection and stabilisation and it was believed that the FTIB could be obtained and the BES closed with the aid of one extra instrument.

4= the technique was planned to require moderately complex intestinal selection and stabilisation and it was believed that the FTIB could be obtained and the BES closed with the aid of greater than one extra instrument.

5= the technique was planned to require markedly complex intestinal selection and stabilisation and it was believed that greater than one extra instrument

and/or complex modification of the instrument or technique would be required to obtain a FTIB and for the BES to be closed.

Histological Assessment

The location of the biopsy varied from mid way between the anti mesenteric and mesenteric border of the intestine and the anti-mesenteric border of the proximal jejunum to the ileum dependent on the opinion of the investigator in the preliminary examinations. All the histologically assessed samples in the study were obtained from the anti-mesenteric border of the distal jejunum and ileum (20cm oral of the ileocaecal junction to 20cm aboral to the ileocaecal fold i.e. the most distal jejunum). The biopsy was retrieved from the instrument or elsewhere with a Brown Adson Forcep, placed in a standard biopsy sampling pot three quarters full with Formalin 10%, numerically labelled and submitted for histological examination. The biopsies were assessed blindly by one European boarded certified histopathologists. Each sample was reported on as follows. First an overall description of the biopsy was outlined; the thickness and the presence or absence of different intestinal layers was commented on. Subsequently the presence or absence of mucosa and its quality, the length of intact mucosa and a written assessment of the villi were recorded. The villous height to crypt height ratio was recorded where it was possible. The length of intact mucosa was recorded in mm. Comments on the submucosa, muscularis layers and serosa include their presence or absence and in the case of the serosal layer the presence of the serosal mesothelium and any disruption or artefact within each individual layer. The presence or absence of submucosal or myenteric plexae and their quality are commented on separately. The opinion of the technician as regards any technical difficulties in histological processing was also recorded. Finally a commentary was presented on the quality of the sample overall and its suitability for histological assessment.

Each biopsy sample was given a score based on the histopathologists opinion of the overall quality of the biopsy sample. The scores ranged from 1 to 6 as follows 1=Excellent, 2=Very Good, 3=Good, 4=Acceptable, 5=Poor, 6= Very Poor. Finally a comment on the overall quality and acceptability of the biopsy and a justification of the score was provided.

Appendix 4. Preliminary investigation 2: Biopsy Excision and Biopsy Closure

Investigation of Biopsy Excision

Identification of possible tasks: biopsy excision techniques

The first limiting factor in the preliminary investigations is the selection of an appropriate biopsy excision technique. The process of excision is defined by the excision method and stabilisation, biopsy shape and size. The author carried out a brainstorming and literature review, and a list of possible methods of biopsy were identified principally these were instruments used to obtain intestinal biopsy, biopsy or veterinary instrumentation. The techniques used to obtain the biopsy samples were divided into different categories. The optimal biopsy size was investigated using the selected technique. It was first proposed that a set number of biopsies at differing lengths would be achieved however in practice the length of the biopsy varied dependent on the size of the intestine and other variables. For this reason all samples were between 5-6mm in length for this preliminary investigation. Two sizes as regards width were examined (2mm and 3mm).

Number	Technique Description	Instruments used
1 Used in a grasping/biting fashion		Ferris Smith Rongeurs
		Uterine forceps
		Rectal biopsy
		Endoscopic biopsy forceps
2 Used in a rotating and stabbing action		Keyes bunch biopsy 8mm
		Tru cut liver biopsy instrument
3 Used on the anti mesenteric border in a cutting or punch action against the wooden bench		Keyes bunch biopsy 8mm
4 Using standard surgical instruments in cutting or sawing action – pinch biopsy		No. 11 scalpel and Brown Adson Forceps
5 Using standard surgical instruments in a scissors cutting action		Brown Adson Forceps and Metzenbaum Scissors

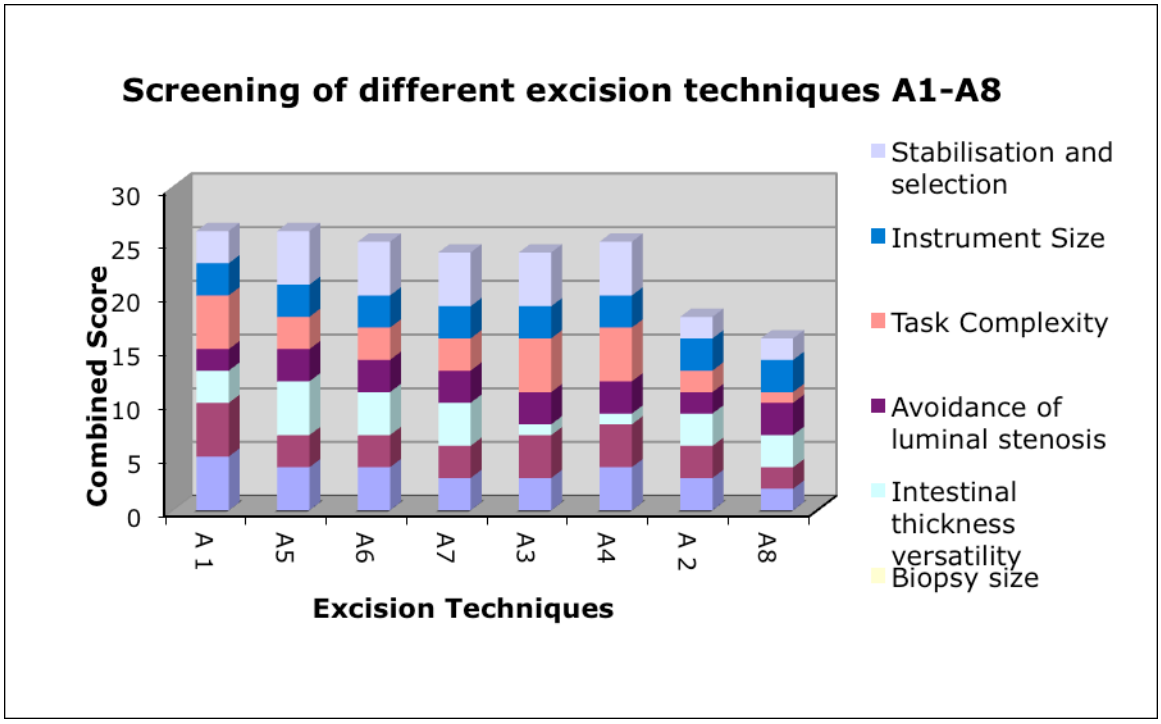
Appendix 4 Table1 A description of the identified methods of biopsy excision. The instruments used were categorised into 5 different techniques.

Screening: Investigation of biopsy excision techniques

The most appropriate techniques 1-5 (Appendix 4. Table1) were examined in a number of shapes labelled A1-A8 (Appendix 4. Table 2). A1-A8 were also scored (Appendix 4. Figure 1) and ranked (Appendix 4. Table 3) using the scoring system outlined in appendix 3. A histological assessment was summarised (Appendix 4.Table4).

Study No.	Size and Shape	Method of excision
A 1	Triangle 5x4mm	Placement of the intestine on a hard surface, stabilisation using bowel forceps at approx. 30 degrees which apposed the bowel wall so as to deform the bowel in a longitudinal direction, an incision is made ("Chopping board-like") through both serosal walls using one no.10 scalpel blade twice.
A 2	Triangle 5x4mm	Placement of the intestine on a hard surface, stabilisation using bowel forceps at approx. 30 degrees which apposed the bowel wall so as to deform the bowel in a longitudinal direction, an incision is made with two blades simultaneously ("Chopping board-like") at an angle in keeping with the proposed size of the biopsy through both serosal walls.
A 3	Rectangle 5x4mm	The bowel was manipulated with a De Bakey forceps and an incision was made through one serosal wall using a scissors
A 4	Rectangle 4x3mm	Placement of the intestine on a hard surface, stabilisation using bowel forceps at approx. 30 degrees which apposed the bowel wall so as to deform the bowel in a longitudinal direction, an incision is made ("Chopping board-like") through both serosal walls three separate times
A 5	Circular 4mm	Intestine manipulated with a De Bakey forceps and a Keyes punch biopsy was pushed/rotated into intestine to incise through one serosal wall
A 6	Circular 6mm	Intestine manipulated with a De Bakey forceps and a Keyes punch biopsy was pushed/rotated into intestine to incise through one serosal wall
A 7	Circular 8mm	Intestine manipulated with a De Bakey forceps and a Keyes punch biopsy was pushed/rotated into intestine to incise through one serosal wall
A 8	Semicircular 8mm	Placement of the intestine on a hard surface, stabilisation using bowel forceps at approx. 30 degrees which apposed the bowel wall so as to deform the bowel in a longitudinal direction, an incision is made using approximately half the circumference (on occasion greater than half the circumference of the circular blade was used) of the 8mm Keyes punch biopsy ("Chopping board-like") through both serosal walls

Appendix 4 Table 2 Evaluation of biopsy excision methods A1-A8



Appendix 4 Figure 1 Bar Chart: Screening of different excision techniques (A1 -A8) using a scoring system

The summary of the results of the histological examination of the A1 – A8 techniques are outlined (Appendix 4 Table 3 and Appendix 4 Table 4). A larger score reflects a more poor quality sample.

Technique Name	A8	A3	A1	A7	A6	A5	A2	A4
Histological Score	7	10	11	11	14	15	15	n/a
Histological Ranking	1st	2nd	3rd	3rd	5th	6th	6th	8th

Appendix 4 Table 3 The histological score and ranking of technique A1-A8

Sample	A1	A2	A3	A4
Description of Biopsy	Full thickness biopsy with variable amount and quality of submucosa and mucosa	Partial thickness lacking mucosa	One sample is partial thickness, otherwise samples are full thickness	No histopathological samples were obtained
Mucosa	For the most part poor quality of mucosa sample	Lacking sufficient mucosa	Substantial mucosa present however variable in quality due to fragmentation and disorganization	
Mucosa (mm)	0-5	0-1	5	
Assessment of villi	Variable amount of mucosa	Lacking sufficient mucosa	Difficulty in assessing the villi	
Submucosa	Splitting/disruption of the submucosa and resultant loss of detail	Submucosa present can be adequately assessed	Intact and can be assessed adequately	
Muscularis Layers	Of adequate quality; oblique sectioning in one sample	Intact	Some splitting of muscularis, diagnostic interpretation is more difficult	
Serosa	Mesothelium has sloughed	Mesothelium has sloughed	Mesothelium has sloughed.	
Submucosal and Myenteric Plexae	Myenteric ganglia visible in one biopsy and submucosal present in one sample	Both submucosal and myenteric ganglia are present	Submucosal and myenteric ganglia are visible in all	
Histology Processing	No technical difficulties	Lack of mucosa	No technical difficulties	
Biopsy Score	2,4,5	5,5,5	4,3,3	
Total Score	11	15	10	
Comment	Some artefacts variable amount of mucosa, variable but adequate muscularis quality	Useful for assessment of muscularis but not of mucosa	Adequate biopsy however some problems with the quality of mucosal sample	

Appendix 4 Table 4 Histological sampling of technique A1 – A4. The results are a synopsis of three samples of each technique. * 1=Excellent, 2=Very Good, 3=Good, 4=Acceptable, 5=Poor, 6= Very Poor (Continued on next page)

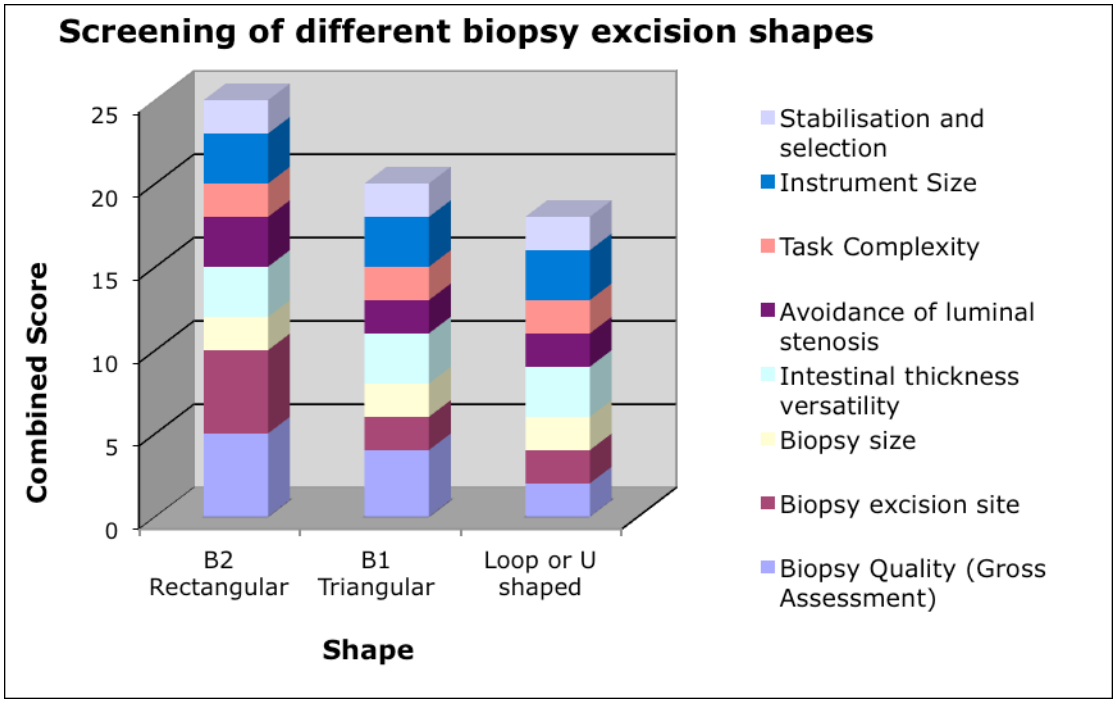
Sample	A5	A6	A7	A8
Description of Biopsy	Full thickness with variable amount and quality of submucosa and mucosa	Partial thickness lacking mucosa	One sample is partial thickness, otherwise samples are full thickness	Full thickness but two folded, one is missing some serosa and muscularis
Mucosa	For the most part poor quality of mucosa sample	Lacking sufficient mucosa	Substantial mucosa present however variable in quality due to fragmentation and disorganization	Mucosal folding
Mucosa (mm)	0-5	0-1	5	2-4mm
Assessment of villi	Variable amount of mucosa	Lacking sufficient mucosa	Difficulty in assessing the villi	Somewhat disrupted, difficulty in crypt ratio measurement
Submucosa	Splitting/disruption of the submucosa and resultant loss of detail	Submucosa present can be adequately assessed	Intact and can be assessed adequately	Submucosa is adequate
Muscularis Layers	Of adequate quality; oblique sectioning in one sample	Intact	Some splitting of muscularis, diagnostic interpretation is more difficult	Present and intact, one of the samples is missing some serosa and muscularis
Serosa	Mesothelium has sloughed	Mesothelium has sloughed	Mesothelium has sloughed.	Mesothelium has sloughed
Submucosal and Myenteric Plexae	Myenteric ganglia visible in one biopsy and submucosal present in one sample	Both submucosal and myenteric ganglia are present	Submucosal and myenteric ganglia are visible in all	Submucosal and myenteric ganglia are present
Histology Processing	No technical difficulties	Lack of mucosa	No technical difficulties	No technical difficulties
Biopsy Score	2,4,5	5,5,5	4,3,3	3,2,2
Total Score	11	15	10	7
Comment	Some artefacts, variable amount of mucosa, variable but adequate muscularis quality	Useful for assessment of muscularis but not of mucosa	Adequate however some problems with the quality of mucosal sample	Acceptable biopsy but quality of mucosa is questionable for villi ratio etc.

Appendix 4 Table 4 (cont.) Histological sampling of technique A5-A8. The results are a synopsis of three samples of each technique. * 1=Excellent, 2=Very Good, 3=Good, 4=Acceptable, 5=Poor, 6= Very Poor

The shape of the biopsy excision using the "Chopping board-like" technique (CBT) was then examined (Appendix 4. Table 5) and scored (Appendix 4. Figure 2). A picture of the prototype instruments are outlined (Appendix 6 Figure 1).

No.	Shape and description	Proposed size of Long axis	Proposed size of Short axis base	Excision Instrument
B1	Triangular	Various ranging from 5-8mm	Various ranging from 5-8mm	"Prototypes Excision Triangular" PET
B2	Rectangular	Various Sizes ranging in size from 7mm	Various sizes ranging in size from 5mm	"Prototypes Excision Rectangular" PER
B3	U-shaped	4mm	1-6mm	Prototypes Excision Loop "1a, 2a, 3a, 4a, 6a"

Appendix 4 Table 5 Different excision shapes investigated



Appendix 4 Figure 2 Bar Chart: Screening of different biopsy excision shapes using a scoring system

B1, B2 and B3 were scored and this was summarised in Appendix 4. Figure 2. B1 resulted in a small area of mucosa excised in keeping with its shape. B2 was not

adequately manufactured and so the corners of the biopsy were not excised completely. B3 resulted in a well-formed and repeatable biopsy sample and was the highest ranked shape in this examination.

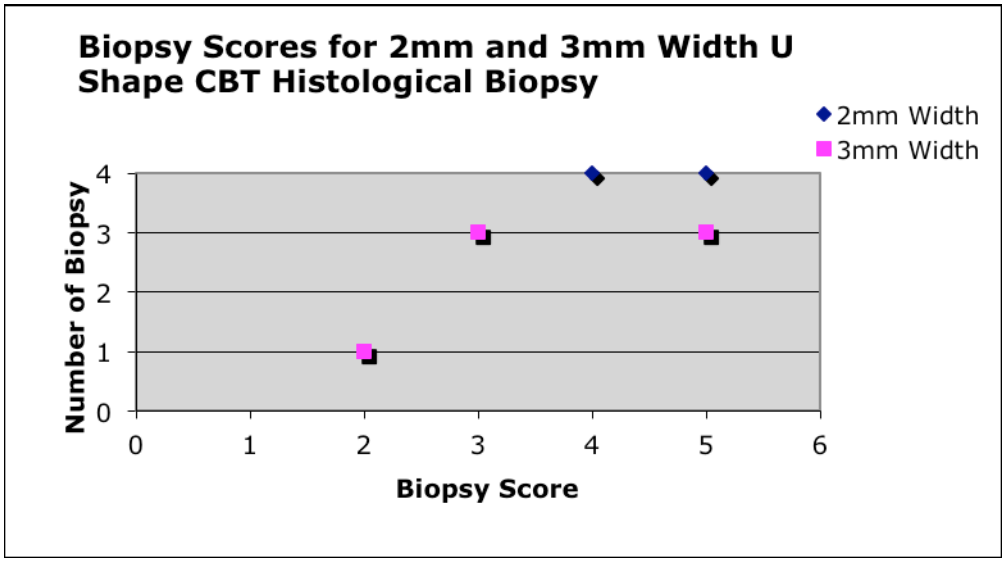
The U-shaped biopsy was trialled using the CBT in 2mm and 3mm width samples (Appendix 4. Table 6). A synopsis of the histological examination are detailed (Appendix 4. Table 7) and the scores are outlined by a graph (Appendix 4. Figure 3).

Proposed size	Stabilisation	Shape
2mm (width) by 5 – 6mm	Needle stabilisation (23 gauge needles positioned placed 2-3mm from the folded intestine border and approximately 1-2mm from the proposed excision site).	U
3mm (width) by 5 – 6mm	Needle stabilisation (23 gauge needles positioned placed 2-3mm from the folded intestine border and approximately 1-2mm from the proposed excision site).	U

Appendix 4 Table 6 Details of the techniques employed in screening of different biopsy sizes

	2mm Width	3mm Width
Full thickness		1
Full thickness but disrupted	2	3
Partial thickness	6	3
Myenteric and Submucosal Ganglia	3	6
Myenteric Ganglia only	4	1
Submucosal Ganglia only	1	
Mucosa Present	4	4
Full thickness of submucosa	4	4
Partial thickness of submucosa	4	2
No submucosa		1
Normal Muscularis	3	2
Split Muscularis	5	4
Disrupted Muscularis		1

Appendix 4 Table 7 Presence of the different intestinal layers of the 2mm and 3mm U-shaped biopsies



Appendix 4 Figure 3 Scatter Plot: Biopsy scores for 2mm and 3mm width U-shaped CBT biopsies

Needle Stabilisation

A technical difficulty encountered with the techniques B1, B2 and B3, which were stabilised using bowel forceps was that the mucosal layers separated from the seromuscular layer of the intestine during downward pressure of the CBT. This resulted in little or no mucosa excised. This idea is illustrated in Appendix 6 Figure 2. A prototype stabilisation forceps fashioned from a sponge forceps was trialled (Appendix 6 Figure 3). It had a barrel shaped end and thus applied pressure to the lumen of the intestine which served to push the mucosal layer closer to the serosal layer as apposed to flattening the layers which occurs when bowel forceps are applied. The most effective stabilisation method in this study was the use of 23 gauge needles outlined in Appendix 6 Figure 4. 23 gauge needles were found to be superior to 25 gauge as the latter deformed and were more difficult to manipulate. It was found that the needles needed to be approximately placed 2-3mm from the folded intestine border and approximately 1-2mm from the proposed excision site. Once the needle stabilisation technique was refined it resulted in successful biopsy (unconfirmed by histology) of a biopsy depth of 4mm and 2-3mm in biopsy width. It was envisaged that the legs of staples advanced before excision would serve a similar function to the needles in allowing the mucosal layer to be excised.

Investigation of Biopsy Closure

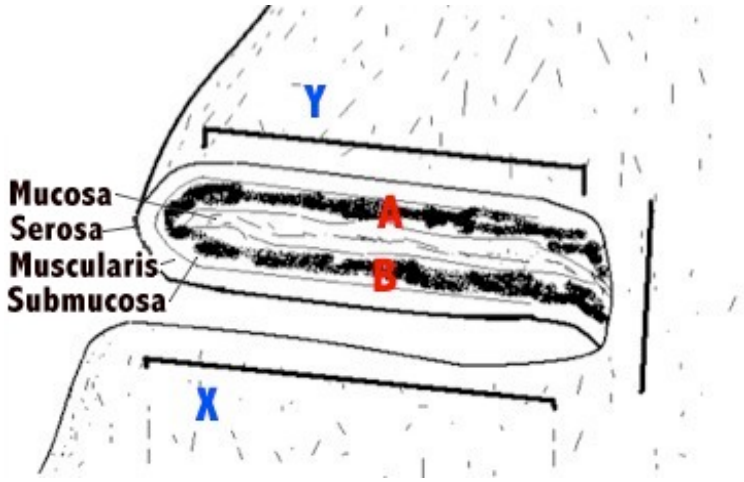
Identification of possible tasks: biopsy closure techniques

The second limiting factor was the BES closure technique. Firstly, the methods that a BES could be closed were outlined (Appendix 4. Figure 4 and Appendix 4. Figure 5). The investigation identified closure methods commonly employed in surgery. It was noted that the BES has effectively 4 walls of intestine. This was illustrated (Figure 4-4 and Figure 4-5). A and B are the folded walls of intestine which are both on the Y and X side of the BES. The biopsy can be closed in the following ways:

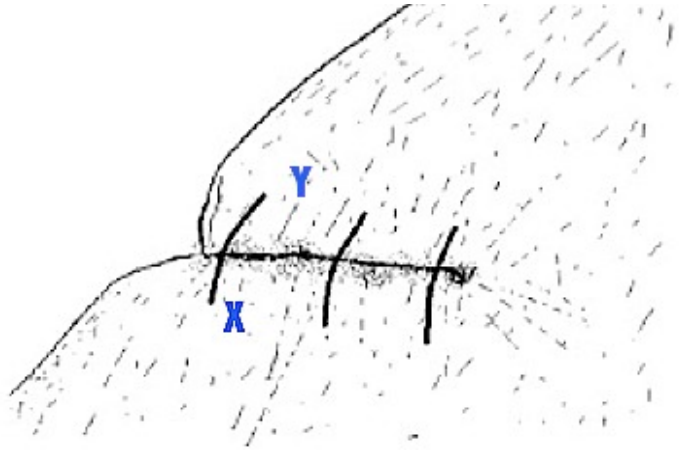
Apposition of A and B walls of both X and Y sides without apposition of X and Y

Apposition of A and B walls of both the X and Y sides with apposition of X and Y

Apposition of the A wall of the X side with the A of the Y side and the apposition of the B of the X side with the B of the Y side



Appendix 4 Figure 4 An illustration of a potential closure method for a U-shape biopsy excision site which does not result in the joining of the X and Y long sides of the biopsy excision site together. Closure was carried out by the apposition of the intestinal walls as illustrated in the apposition of A and B intestinal walls.



Appendix 4 Figure 5 An illustration of a potential closure method for a U-shape biopsy excision site which does result in the joining of the X and Y long sides of the biopsy excision site. It was conceivable that the A and B intestinal walls as illustrated in Appendix 4 Figure 4 could be apposed or similarly not apposed in a closure method of this kind.

The possible closure techniques were compiled after consideration of the ways the BES could be apposed. The double layer of staples was defined as being similar to a linear stapler configuration but configured to a U-shape. The novel staple configuration was any novel staple configuration or staple type to close the excision site in any number of ways. *Skin staples* (Proximate Plus MD 35w - Ethicon Endosurgery) were used to perform anastomosis of small intestine in the horse and dog (Coolman 2000; Gandini and Bertuglia 2006). *Multifire Endohernia*® (Ethicon Endosurgery) - This a laparoscopic instrument used in human medicine to close inguinal hernias. *Suture closure* was achieved by the use of standard hand held instrument using 3 metric Biosyn™ (Covidien plc.) in a two layer continuous Lembert suture pattern. Biosyn™ is a synthetic absorbable suture prepared from a synthetic polyester composed of glycolide (60%), dioxanone (14%), and trimethylene carbonate (26%).

Screening: Investigation of biopsy excision closure techniques

The identified techniques were screened and ranked (Appendix 4. Table 8) using the scoring system (appendix 3).

	Double layer of staples	Novel Staple Configuration	Intra-corporeal suturing	Endoloop®	Skin staples	Endohernia®
Biopsy Quality (Gross Assessment)	n/a	n/a	n/a	n/a	n/a	n/a
Biopsy excision site	n/a	n/a	n/a	n/a	n/a	n/a
Biopsy size	n/a	n/a	n/a	n/a	n/a	n/a
Intestinal thickness versatility	3	3	1	1	3	3
Avoidance of luminal stenosis	2	2	2	2	1	1
Task Complexity	1	2	5	5	5	5
Instrument Size	3	3	5	5	3	3
Stabilisation and selection	2	2	5	5	3	3
Total	11	12	18	18	15	15
	1st	2nd	5th	5th	3rd	3rd

Appendix 4 Table 8 Screening of the potential closure method

Intraluminal Bursting Pressures

The most appropriate techniques were evaluated using intraluminal bursting pressures. The biopsy excision sites were located between the mesenteric and anti-mesenteric border of the distal jejunum and ileum (20cm oral of the ileocaecal junction to 100cm aboral to the ileocaecal fold) and positioned approximately halfway along a section of intestine measuring approximately 42cm in length. A modified water bath (Appendix 6) was fashioned with an ingress connector attached to a water pump and an egress connector attached to a pressure gauge. Jubilee clips were used to attach the intestine to the ingress and egress connectors. The bath was filled with tap water (approximately 22°C). The water pump pushed iodine stained fluid (0.5% solution) via the ingress at a rate of 2l/min into the intestine. The water bath was monitored for signs of leakage of the brown stained fluid. The pressure within the intestine was monitored and if there was a sudden drop in intraluminal pressure then the maximum pressure achieved was recorded. If there was leakage of fluid then the pressure at which this occurred was recorded as the bursting pressure. Luminal reduction at the biopsy site was expressed as a percentage of the control tissue lumen dimensions. The control lumen dimensions were calculated from 3cm and 6cm oral and aboral from the biopsy site (Nieto 2006). A vernier calliper was used to measure the lumen of the intestine. Four bursting pressures were obtained for each closure method tested. The results of the bursting pressures are presented in the results section outlining Prototype Instrument 3 (Figure 4-12 and Table 4-1).

Appendix 5. Results of histological samples - Prototype Instrument 2

Sample Number	Description of Biopsy	Mucosa	Length of Intact Mucosa	Assessment of villi	Submucosa	Muscularis layers	Serosa	Submucosal and myenteric Plexus	Peyer's Patches	Histology Processing	Biopsy Score	Comments
1	Partial thickness biopsy including serosa, muscularis layers, full thickness layer of the submucosa, muscularis mucosa and serosa, but no mucosa. The biopsy is folded, with the submucosa on the inner aspect of the fold.	No interpretable mucosa is present. There are a few fragments of epithelium on the inner fold of the tissue.	No mucosa is present.	Villi cannot be assessed.	The submucosa has some areas of disruption but can be assessed adequately.	Inner circular and outer longitudinal muscularis layers are present and adequately represented.	The serosa and subserosal connective tissue is intact and can be assessed adequately.	Myenteric ganglia are visible but there are no subserosal ganglia.	No Peyer's patches are visible.	The technician had a small amount of difficulty orientating the tissue section.	2	This biopsy is not acceptable for diagnostic interpretation of mucosal changes, but could be of value in assessing changes in the submucosa, muscularis layers and serosa.
2	A full thickness biopsy including mucosa, submucosa, muscularis layers and serosa.	An adequate length of largely intact mucosa is present. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	5mm	Villi are adequately presented, either in longitudinal or oblique orientation. Tips of a few villi are mildly disrupted. The ratio of villous height to crypt depth is 3:1 to 2:1.	The submucosa is folded has some areas of mild disruption, but can be assessed adequately.	Adequate amounts of inner circular and outer longitudinal muscularis layers are present. There is some separation of muscle fibre bundles.	Adequate amounts of intact serosa and subserosal connective tissue are present.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had no difficulty orientating the tissue section.	2	This biopsy is acceptable for most diagnostic purposes.

3	A full thickness folded biopsy including mucosa, submucosa, muscularis layers and serosa, but with disruption of the submucosa and muscularis layers.	An adequate length of folded, mostly intact mucosa is present. Villi are sectioned in longitudinal, oblique or transverse orientation. There is mild disruption of the tips of some villi. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	6mm	Villi are sectioned in longitudinal, oblique or transverse orientation. There is mild disruption of the tips of some villi. The ratio of villous height to crypt depth cannot be determined accurately but appears to be 3:1 to 2:1.	The submucosa has a substantial degree of disruption, which prevents adequate assessment.	Adequate amounts of inner circular and outer longitudinal muscularis layers are present and interpretation is possible, but there is substantial disruption of muscularis layers in some areas of the section. Separation of muscle fibre bundles is also evident.	Adequate amounts of intact serosa and subserosal connective tissue are present.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had a small amount of difficulty orientating the tissue section.	3	This biopsy is acceptable for most diagnostic purposes but there is considerable disruption of tissues in some areas and the orientation of the mucosa prevents full interpretation of villous structure.
4	A full thickness biopsy with largely intact mucosa and submucosa, but some disruption and loss of muscularis layers and serosa.	A substantial amount of interpretable mucosa is present. Most villi are sectioned longitudinally. The tips of some villi are disrupted. There are mild to moderate infiltrates of lymphocytes, plasma cells and eosinophils in the lamina propria.	10mm	Villi can be assessed adequately. The ratio of villous height to crypt depth is 4:1 to 3:1. The tips of some villi are disrupted.	The submucosa is intact and can be assessed adequately. Mild oedema is evident.	Inner circular and outer longitudinal muscularis layers are present. There is disruption and loss of some layers of muscle, with a relatively small amount of the outer circular muscularis layer being present.	A short segment of intact serosa and subserosal connective tissue is available for assessment.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had no difficulty orientating the tissue section.	3	This biopsy is acceptable for most diagnostic purposes although the representation of some portions of the outer muscularis and serosal layers is limited.
5	A full thickness but disrupted biopsy in two pieces with a small amount of folded mucosa attached to one portion of tissue.	A small portion of interpretable mucosa is attached to one portion of tissue. Villi are distorted and many are orientated obliquely or transversely. There are mild to moderate infiltrates of lymphocytes, plasma cells and eosinophils in the lamina propria.	3mm	Villi are distorted and many are in a transverse or oblique orientation. Some have disrupted tips.	A small amount of slightly distorted submucosa is present but it is possible to undertake an adequate assessment.	Inner circular and outer longitudinal muscularis layers are present and are adequately orientated. There is a mild degree of splitting of muscle layers.	The serosa and subserosal connective tissue are largely intact and can be assessed adequately.	A few myenteric ganglia are visible but no submucosal ganglia are visible.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section.	4	This biopsy is acceptable for some diagnostic purposes but the amount of intact mucosa available for diagnostic interpretation is small.

6	A partial thickness biopsy consisting of muscularis layers and serosa but no mucosa or submucosa.	No mucosa is present.	No mucosa is present.	Villi cannot be assessed.	No submucosa is present.	The outer longitudinal and part of the inner circular muscularis layers are present. There is a mild degree of splitting of muscle layers. The muscle tissue is adequately represented.	The serosa and subserosal connective tissue are intact and can be assessed adequately.	Myenteric ganglia are visible but no submucosal ganglia are visible.	No Peyer's patches are visible.	The technician had a small amount of difficulty orientating the tissue section.	5	This biopsy is not acceptable for diagnostic interpretation of mucosal or submucosal changes, but could be of value in assessing changes in the muscularis layers and serosa.
7	Four full or partial thickness portions of intestine with a degree of folding and distortion are present in the histological section, including interrupted segments of mucosa and submucosa, along with muscularis layers and serosa. A small amount of crush artefact is present.	The mucosa is interrupted and some segments are folded or distorted. Tips of many villi are disrupted and there is loss of epithelial cells from the tips and sides of many villi. Some villi are sectioned in a transverse or oblique orientation. Mild to moderate infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	14mm	Tips of many villi are disrupted and there is loss of epithelial cells from the tips and sides of many villi. Some villi are sectioned in a transverse or oblique orientation. It is difficult to assess the ratio of villous height to crypt depth, but it appears to be 2:1 to 1:1.	Much of the submucosa is disrupted; there is only a small amount of intact submucosa.	Relatively small amounts of intact inner circular and outer longitudinal muscularis layers are represented. Some distortion is evident. There is moderate separation of muscle fibre bundles.	Relatively small amounts of subserosal connective tissue are present and there is little intact serosa.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section.	4	This biopsy is acceptable for many diagnostic purposes although some portions of the tissue are separated, distorted and disrupted.
8	A full thickness folded biopsy including a short segment of folded, crushed mucosa, along with submucosa, muscularis layers and serosa.	A short segment of largely disrupted mucosa is present on the inner fold of the tissue sample and the amount of interpretable mucosa is limited. Villi cannot be assessed. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	2mm	Villi cannot be assessed.	The submucosa is folded and has some areas of disruption, but can be assessed adequately.	Adequate amounts of inner circular and outer longitudinal muscularis layers are present. There is some separation of muscle fibre bundles and disruption is evident in some areas.	Adequate amounts of intact serosa and subserosal connective tissue are present.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had a small amount of difficulty orientating the tissue section.	5	This biopsy is not acceptable for diagnostic interpretation of mucosal changes, but could be of value in assessing changes in the submucosa, muscularis layers and serosa.

9	The section contains one full thickness portion of intestine and two partial thickness fragments. Adequate segments of mucosa, submucosa, muscularis layers and serosa are represented.	An adequate length of largely intact mucosa is present. There is mild disruption of the tips of some villi. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	6mm	Villi are sectioned mostly in longitudinal orientation and can be assessed adequately. There is mild disruption of the tips of some villi. The ratio of villous height to crypt depth is 3:1 to 2:1.	There is some separation of collagen fibre bundles in the submucosa, but overall the submucosa can be assessed adequately.	Adequate amounts of inner circular and outer longitudinal muscularis layers are present. There is some separation of muscle fibre bundles.	Moderate amounts of intact serosa and subserosal connective tissue are present.	Submucosal ganglia are present but no myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had no difficulty orientating the tissue section.	3	This biopsy is acceptable for most diagnostic purposes although there is some separation of the tissue and no myenteric ganglia are visible.
10	Three full thickness but folded and distorted portions of intestine are present in the histological section, including interrupted segments of mucosa and submucosa, along with muscularis layers and serosa.	The mucosa is interrupted and some segments are folded or distorted. Tips of many villi are disrupted. Other villi are sectioned in an oblique orientation. Mild to moderate infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	12mm	Villi appear to be relatively short but it is not possible to assess the ratio of villous height to crypt depth.	Most of the submucosa is intact and can be assessed adequately. Some areas are disrupted or distorted.	Inner circular and outer longitudinal muscularis layers are adequately represented, although some areas are distorted.	The serosa and subserosal connective tissue are intact and can be assessed adequately.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section.	3	This biopsy is acceptable for most diagnostic purposes although some portions of the tissue are distorted.
11	A full thickness but disrupted and distorted biopsy including short segments of mucosa and submucosa, along with muscularis layers and serosa.	A short segment of intact but folded and distorted mucosa is present. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	2mm	Villi are mostly sectioned in oblique orientation. The ratio of villous height to crypt depth cannot be determined accurately but appears to greater than 2:1.	A small portion of partly disrupted submucosa is present. The submucosa cannot be adequately assessed.	Adequate amounts of inner circular and outer longitudinal muscularis layers are present, but there is distortion, disruption and separation of muscle fibre bundles in many areas.	Discontinuous segments of serosa and subserosal connective tissue are present.	Myenteric ganglia are present but no submucosal ganglia are visible.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section.	5	This biopsy is not acceptable for diagnostic interpretation of mucosal changes, but could be of value in assessing changes in the muscularis layers.
12	A partial thickness biopsy consisting of disrupted muscularis layers with submucosa and serosa but no mucosa.	No mucosa is present.	No mucosa is present.	Villi cannot be assessed.	Submucosa is present but is not orientated optimally.	Inner circular and outer longitudinal muscularis layers are present, but are disrupted and not well orientated.	A small amount of serosa and subserosal connective tissue are present.	Submucosal ganglia are visible but no myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section.	5	This biopsy is not acceptable for diagnostic interpretation of mucosal changes, but could be of value in assessing some changes in the submucosa, muscularis layers and serosa.

13	A full thickness folded biopsy including short segments of mucosa and submucosa, along with muscularis layers and serosa.	A substantial amount of interpretable mucosa is present. Most villi are sectioned longitudinally. The tips of some villi are disrupted. There are mild to moderate infiltrates of lymphocytes, plasma cells and eosinophils in the lamina propria.	10mm	Villi can be assessed adequately. The ratio of villous height to crypt depth is 4:1 to 3:1. The tips of some villi are disrupted.	The submucosa is intact and can be assessed adequately. Mild oedema is evident.	Inner circular and outer longitudinal muscularis layers are present. There is a mild separation of some muscle fibre bundles, but the muscle is adequately represented.	The serosa and subserosal connective tissue are largely intact and can be assessed adequately.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had no difficulty orientating the tissue section.	2	This biopsy is acceptable for most diagnostic purposes.
14	The section contains one full thickness portion of intestine and three partial thickness fragments. Short segments of mucosa are represented, along with submucosa, muscularis layers and serosa are represented.	The mucosa is distorted, but there is sufficient for interpretation. Villi are sectioned in oblique or transverse orientation. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	5mm	Villi are sectioned oblique or transverse orientation and can be assessed with partial adequacy. The ratio of villous height to crypt depth cannot be determined accurately but appears to be greater than 2:1.	There is considerable disruption of the submucosa, which reduces the ability to interpret findings adequately.	Adequate amounts of inner circular and outer longitudinal muscularis layers are present. There is some separation of muscle fibre bundles.	Adequate amounts of intact serosa and subserosal connective tissue are present.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section.	3	This biopsy is acceptable for most diagnostic purposes although there is some separation and distortion of the tissue.
15	Full thickness biopsy including mucosa, submucosa, muscularis layers and serosa but the mucosa and submucosa are separated from the muscularis layers.	A substantial amount of interpretable mucosa is present. Most villi are sectioned transversely or obliquely. Mild to moderate infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria. There is disruption of some villi.	10mm	A few villi can be assessed adequately, whereas most are sectioned transversely or obliquely and there is disruption of some villi.	The submucosa is intact and can be assessed adequately. Congestion and mild oedema are evident in the submucosa.	Inner circular and outer longitudinal muscularis layers are present and adequately represented.	The serosa and subserosal connective tissue is intact and can be assessed adequately.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had a small amount of difficulty orientating the tissue section.	2	This biopsy is acceptable for most diagnostic purposes.

16	A full thickness intestinal biopsy including substantial amounts of mucosa and submucosa, along with smaller amounts of inner circular and outer longitudinal muscularis layers and serosa.	A substantial length of intact mucosa is present, but there is the appearance of folding of mucosa due to tangential sectioning across the underlying submucosa. There is disruption of the tips of a number of villi. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	10mm	Villi are sectioned mostly in oblique or transverse orientation. There is disruption of the tips of some villi. The ratio of villous height to crypt depth cannot be determined accurately but appears to be 3:1 to 2:1.	The submucosa is sectioned tangentially and has some areas of separation related to folding of the mucosa but mostly can be assessed adequately.	Small amounts of inner circular and outer longitudinal muscularis layers are present. There is some separation of muscle fibre bundles.	Small amounts of intact serosa and subserosal connective tissue are present.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had a small amount of difficulty orientating the tissue section.	3	This biopsy is acceptable for most diagnostic purposes but the orientation of the mucosa prevents full interpretation of villous structure.
17	Partial thickness biopsy including serosa, muscularis layers and a small amount of submucosa, but no mucosa.	No mucosa is present.	No mucosa is present.	Villi cannot be assessed.	Only a small portion of submucosa is visible and therefore overall the submucosa cannot be assessed adequately.	Inner circular and outer longitudinal muscularis layers are present and adequately represented.	The serosa and subserosal connective tissue is intact and can be assessed adequately.	Myenteric ganglia are present but no submucosal ganglia are visible.	No Peyer's patches are visible.	The technician had a small amount of difficulty orientating the tissue section.	5	This biopsy is not acceptable for diagnostic interpretation of mucosal changes, but could be of value in assessing changes in the muscularis layers and serosa.
18	The section contains two partial thickness portions of intestine with a short segment of folded, distorted mucosa lining one portion of tissue.	A short segment of mucosa is present and the amount of interpretable mucosa is limited. The tips of villi are disrupted. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	3mm	Villi appear to be relatively short but it is not possible to determine the ratio of villous height to crypt depth. The tips of villi are disrupted.	Only a small amount of distorted submucosa is present; it is not possible to assess the submucosa adequately.	Relatively small portions of inner circular and outer longitudinal muscularis layers are present, but the tissue is not well orientated in the histological section.	A small amount of serosa and subserosal connective tissue is present, but it is largely disrupted and difficult to interpret.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had a small amount of difficulty orientating the tissue section.	4	This biopsy is acceptable for many diagnostic purposes but the amount of mucosa available for diagnostic interpretation is relatively small.

19	A full thickness mildly folded biopsy including mucosa and submucosa, along with partly disrupted muscularis layers and serosa.	An adequate length of interpretable mucosa is present. Villi are relatively short. There is some disruption of the tips of villi. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	8mm	Villi appear to be relatively short. There is disruption of the tips of some villi. The ratio of villous height to crypt depth is 2:1 to 1:1.	There is moderate disruption of the submucosa, but the histological appearance can be assessed adequately.	Inner circular and outer longitudinal muscularis layers are present. There is a moderate degree of splitting and separation of muscle fibre bundles.	Segments of the serosa and subserosal connective tissue are disrupted, but there is sufficient tissue to allow adequate assessment.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had no difficulty orientating the tissue section.	3	This biopsy is acceptable for most diagnostic purposes although there is some disruption of the submucosa, muscularis layers and serosa.
20	A full thickness slightly folded biopsy including mucosa and submucosa, along with muscularis layers and serosa.	A moderate length of largely intact mucosa is present. There is disruption of the mucosa on the inner fold of the tissue and also disruption of the tips of a number of villi elsewhere. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	6mm	Villi are sectioned mostly longitudinally, but the tips of many villi are disrupted. The ratio of villous height to crypt depth is 3:1 to 2:1.	The submucosa is partially disrupted but can be interpreted adequately.	Substantial amounts of inner circular and outer longitudinal muscularis layers are present. There is some separation of muscle fibre bundles.	Some segments of serosa and subserosal connective tissue are disrupted, but adequate amounts of intact tissue are available for interpretation.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had no difficulty orientating the tissue section.	3	This biopsy is acceptable for most diagnostic purposes but there is some disruption of villous tips.
21	A full thickness folded biopsy including mucosa, submucosa, muscularis layers and serosa. The biopsy is distorted due to twisting.	A substantial amount of interpretable mucosa is present. Most villi are sectioned longitudinally. The tips of some villi are disrupted. There are mild infiltrates of lymphocytes, plasma cells and eosinophils in the lamina propria.	9mm	Villi can be assessed adequately. The ratio of villous height to crypt depth is 4:1 to 3:1. The tips of some villi are disrupted.	The submucosa is intact and can be assessed adequately. Mild disruption is evident in one area.	Inner circular and outer longitudinal muscularis layers are present. There is a mild separation of some muscle fibre bundles, but the muscle is adequately represented.	The serosa and subserosal connective tissue are intact and can be assessed adequately.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section due to twisting of the biopsy specimen.	2	This biopsy is acceptable for most diagnostic purposes.

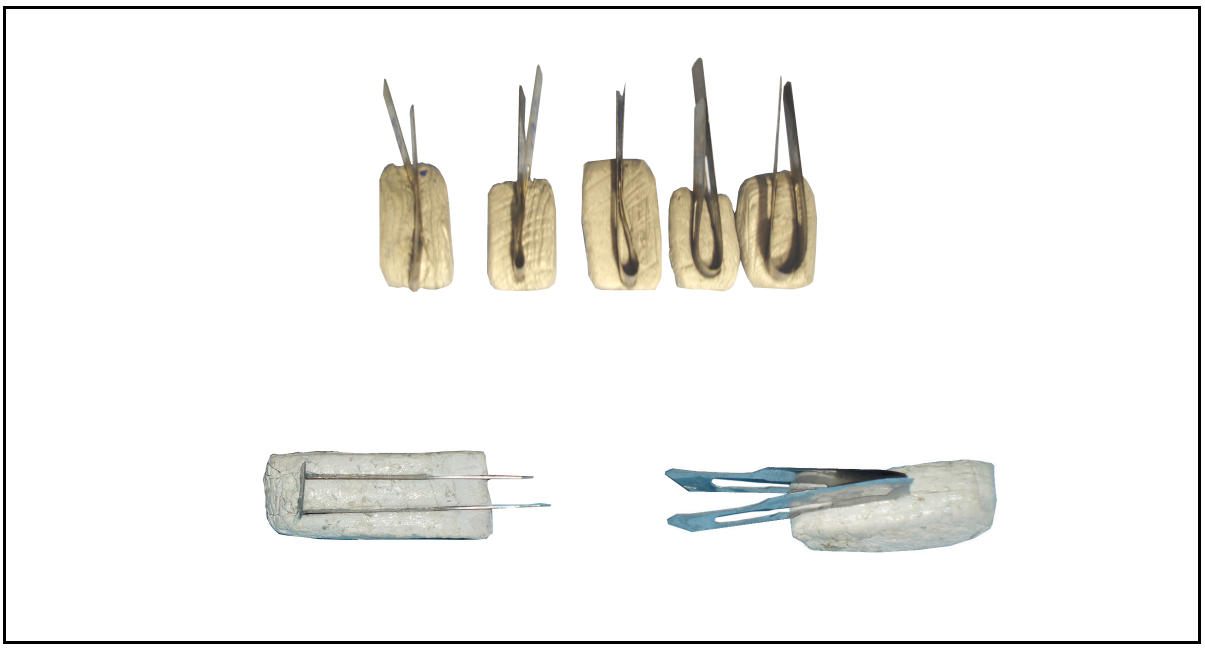
22	A full thickness but disrupted biopsy in two pieces with a small amount of mucosa attached to subserosa and obliquely orientated muscularis layers and serosa.	A small portion of interpretable mucosa is attached to one portion of tissue. Villi are disrupted. Fragments of epithelium lie adjacent to the other portion of tissue. There are mild infiltrates of lymphocytes, plasma cells and eosinophils in the lamina propria.	1mm	The section contains a small number of villi with disrupted tips.	Only a small amount of submucosa is present and this is distorted. Limited assessment can be undertaken.	Inner circular and outer longitudinal muscularis layers are present but are not well orientated. There is a mild degree of splitting of muscle layers.	The serosa and subserosal connective tissue are largely intact and can be assessed adequately.	A few submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section.	4	This biopsy is acceptable for some diagnostic purposes but the amount of mucosa and submucosa available for diagnostic interpretation is small.
23	A disrupted full thickness biopsy including a short segment of mucosa, fragmented submucosa and two portions of partially disrupted muscularis layers and serosa separate from the mucosa.	A small amount of interpretable mucosa is present. Much of the remaining mucosa has been denuded to the level of the muscularis mucosa. The remaining mucosa is folded and the tips of villi are eroded and disrupted. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	1.5mm	Partial assessment of villi is possible. There is disruption of the tips of villi.	The submucosa is disrupted and therefore only a partial assessment can be made.	Inner circular and outer longitudinal muscularis layers are present. The muscularis layers are separated from the mucosa and submucosa. There is some disruption of the inner circular layer, but the muscle tissue is adequately represented.	There is some disruption of the serosa and subserosal connective tissue, but some degree of assessment is possible.	Submucosal and myenteric ganglia are visible, although the myenteric plexus is represented by only a small number of neurones.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section due to disruption of the specimen.	4	This biopsy does not contain enough intact mucosa to be fully acceptable for diagnostic interpretation of mucosal changes. The submucosa, muscularis layers and serosa are present, but the diagnostic value of these tissues is reduced slightly due to disruption.
24	Partial thickness biopsy including muscularis layers and serosa, but no mucosa or submucosa.	No mucosa is present.	No mucosa is present.	Villi cannot be assessed.	No submucosa is present.	Inner circular and outer longitudinal muscularis layers are present but are distorted and sectioned in an oblique plane. There is mild splitting of layers.	Serosa and subserosal connective tissue are mildly distorted, but are adequately represented.	Myenteric ganglia are present but no submucosal ganglia are visible.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section.	5	This biopsy is not acceptable for diagnostic interpretation of mucosal changes, but could be of value in assessing changes in the muscularis layers.

25	A full thickness biopsy including a short segment of mucosa, submucosa and Peyer's patch, along with muscularis layers and serosa.	A relatively short segment of mucosa with some disruption of villi is present. A Peyer's patch is present, with several lymphoid foci and formation of a mound lined by epithelial cells. Mild infiltrates of lymphocytes and plasma cells and eosinophils are present in the lamina propria.	4mm	There is a moderate degree of disruption of villi, but most can be assessed with reasonable adequacy. The ratio of villous height to crypt depth is 3:1.	The submucosa is largely intact and can be assessed adequately. There is some pinching and disruption of the submucosa at the edges of the tissue, with fragments of mucosa carried over into these areas.	Inner circular and outer longitudinal muscularis layers are present and are largely intact.	The serosa and subserosal connective tissue are intact and can be assessed adequately.	Submucosal and myenteric ganglia are visible.	A small Peyer's patch is present, consisting of several lymphoid foci with formation of a mound lined by modified epithelium ("M" cells).	The technician had no difficulty orientating the tissue section.	3	This biopsy is acceptable for most diagnostic purposes although the length of interpretable mucosa is relatively short and there is disruption of some villi.
26	A full thickness folded biopsy including folded and distorted mucosa and submucosa, along with muscularis layers and serosa.	The mucosa is folded and distorted. There is some distortion of villi, many of which are in oblique or transverse orientation. Mild to moderate infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	9mm	Villi are distorted and many are in a transverse or oblique orientation. Some have disrupted tips. The ratio of villous height to crypt depth is 4:1 to 3:1.	The submucosa is distorted and slightly disrupted but is assessable.	Inner circular and outer longitudinal muscularis layers are present. There is a mild splitting and disruption of layers, but the muscle tissue is adequately represented.	The serosa and subserosal connective tissue are intact and can be assessed adequately.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had a small amount of difficulty orientating the tissue section.	3	This biopsy is acceptable for many diagnostic purposes but the mucosa and submucosa are distorted.
27	Partial thickness biopsy including mucosa and submucosa but no muscularis layers or serosa.	A substantial amount of interpretable mucosa is present. Most villi are sectioned longitudinally or obliquely; in some areas the villi are sectioned transversely. There is some erosion of the mucosal surface and some disruption of villi. Mild to moderate infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	17mm	Villi cannot be assessed.	The submucosa is intact and can be assessed adequately. Mild oedema is evident.	Inner circular and outer longitudinal muscularis layers are present and adequately represented.	No serosa or subserosal connective tissue is present.	Submucosal ganglia are visible but no myenteric ganglia are visible due to the absence of the muscularis layers from the histological section.	No Peyer's patches are visible.	The technician had little difficulty orientating the tissue section.	4	This biopsy is acceptable for diagnostic interpretation of mucosal and submucosal changes, but cannot be used to assess changes in the muscularis layers or serosa.

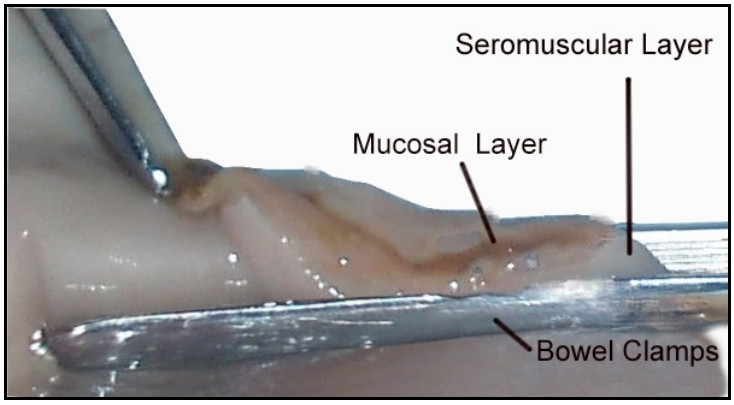
28	A full thickness but distorted biopsy with a small amount of mucosa attached by a narrow band of submucosa to obliquely orientated muscularis layers and serosa.	A small amount of poorly orientated mucosa is present. Villi are sectioned in oblique and transverse orientation. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	3mm	Only partial assessment of villi is possible, since only a small portion of mucosa is available for assessment and most villi are sectioned obliquely or transversely.	Only a small amount of submucosa is present and this is partially disrupted. Limited assessment can be undertaken.	Inner circular and outer longitudinal muscularis layers are present but are distorted and sectioned in an oblique plane. There is mild splitting of layers, but the muscle tissue is adequately represented.	The serosa and subserosal connective tissue are largely intact and can be assessed adequately.	Myenteric ganglia are visible but there are no subserosal ganglia.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section due to partial disruption of the specimen.	3	This biopsy is acceptable for many diagnostic purposes but the amount of mucosa and submucosa available for diagnostic interpretation is relatively small and there is some distortion of the muscularis layers.
29	A full thickness slightly folded biopsy including mucosa, submucosa, muscularis layers and serosa.	An adequate length of interpretable mucosa is present, but there is disruption of the tips of numerous villi. Mild infiltrates of lymphocytes, plasma cells and eosinophils are present in the lamina propria.	8mm	Villi are sectioned mostly in longitudinal orientation. There is disruption of the tips of many villi. The ratio of villous height to crypt depth appears to be 3:1 to 2:1.	The submucosa has some areas of disruption, but can be assessed adequately.	Adequate amounts of inner circular and outer longitudinal muscularis layers are present. There is some separation of muscle fibre bundles and disruption is evident in some areas.	Adequate lengths of intact serosa and subserosal connective tissue are present.	Submucosal and myenteric ganglia are visible.	No Peyer's patches are visible.	The technician had no difficulty orientating the tissue section.	3	This biopsy is acceptable for most diagnostic purposes but there is some disruption of villous tips.
30	Partial thickness biopsy including muscularis layers and small amounts of distorted submucosa and subserosa, but no mucosa.	No mucosa is present.	No mucosa is present.	Villi cannot be assessed.	Only a small portion of submucosa is visible, but is not well orientated and overall the submucosa cannot be assessed adequately.	Inner circular and outer longitudinal muscularis layers are present but are distorted and sectioned in an oblique plane. There is mild splitting of layers.	A small amount of distorted subserosal connective tissue is present, so overall the serosa cannot be assessed adequately.	Myenteric ganglia are present but no submucosal ganglia are visible.	No Peyer's patches are visible.	The technician had some difficulty orientating the tissue section.	5	This biopsy is not acceptable for diagnostic interpretation of mucosal changes, but could be of value in assessing changes in the muscularis layers.

Appendix 5 Table 1 Histological detail of biopsies 1-30 excised by Prototype Instrument 2

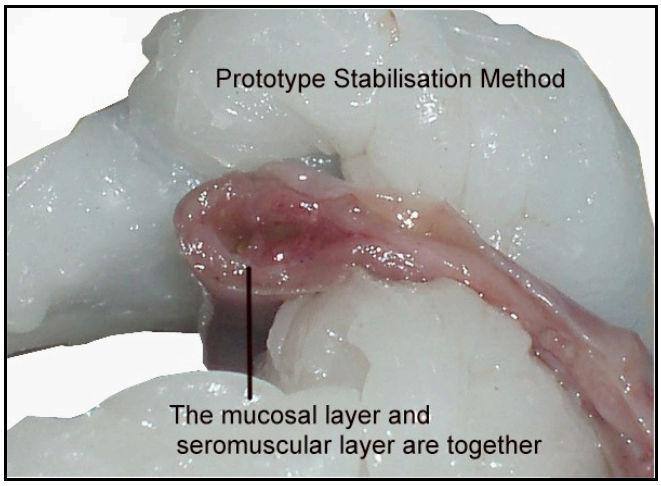
Appendix 6. Figures of equipment and techniques



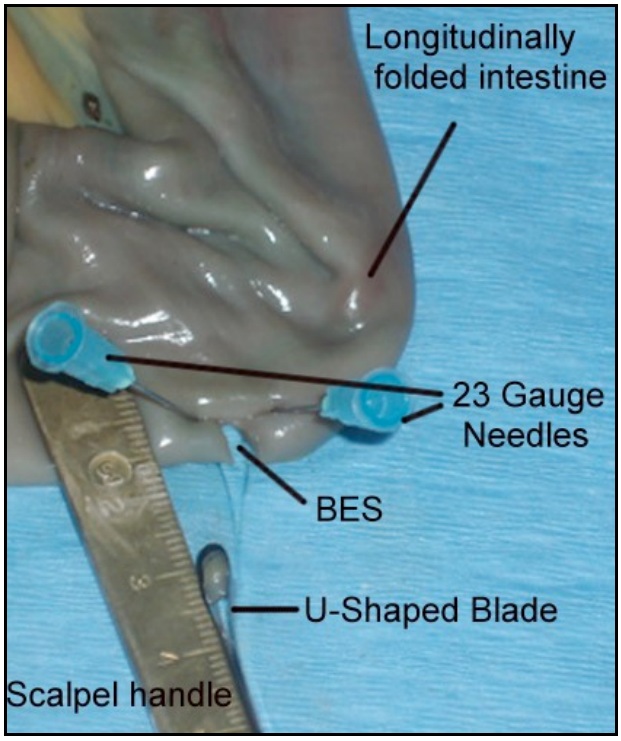
Appendix 6 Figure 1 "Prototype Excision Triangular", "Prototype Excision Rectangular" and "Loop Excision Blades 1a, 2a, 3a, 4a, 5a" are illustrated. The blades are fashioned from no. 22 scalpel blades (Swann Morton Ltd., UK) in the case of the "Prototype Excision Triangular" and "Prototype Excision Rectangular" bottom right and bottom left respectively. The loop blades (top) have been fashioned from regular feather microtome blades (Surgipath Ltd., UK), which have been bent to the desired shape and size with the aid of a Bunsen burner in the case of the "Loop Excision Blades 1a, 2a, 3a, 4a, 5a". The blades are anchored in a synthetic metal composite, which could be moulded to the required shape to form a base. The base was required to be robust as there was a large amount of downward pressure required. The prototype instruments are a small selection of the prototype instruments used in the investigation of the different excision shapes.



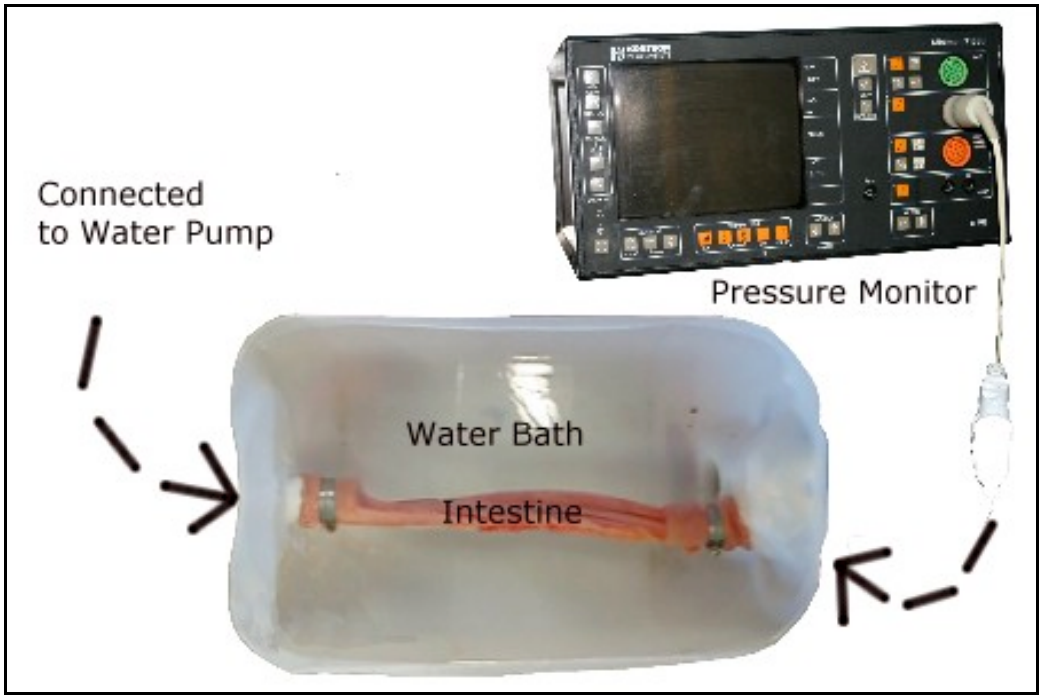
Appendix 6 Figure 2 Bowel clamps were applied to jejunum before transection. It was apparent that the seromuscular layer and mucosal layer have slid away from each other. It was for this reason that a method of stabilisation was required to ensure that the layers do not slip away from the excision method used in a CBT.



Appendix 6 Figure 3 Transected section of jejunum within a sponge forceps which was modified to encompass a plastic rounded edge; this was used to push the mucosal layer closer to the seromuscular layer. The technique was abandoned due to difficulties in excising the biopsy as the plastic end was cumbersome and the technique was not deemed to be as efficient as the needle stabilisation.



Appendix 6 Figure 4 Longitudinally folded section of intestine in which a U-shaped full thickness intestinal biopsy was excised whilst the intestine was stabilised using needle stabilisation. The full thickness intestinal biopsy was within the u-shaped blade. An outline of the U-shaped blade was created in the paper drape by the downward pressure used in the "chopping board-like" technique.



Appendix 6 Figure 5 Experimental setup for the determination of the intraluminal bursting pressures. 50ml syringes (BD, USA) were cross-sectioned at the 30ml marker and the outer casing was used to attach the intestine by the aid of Jubilee clips, (Multiband 11mm Zinc Plated Mild Steel, L. Robinson & Company). Holes were made in which the syringes were positioned at the top and the bottom of a plastic drum (5 gallon drum, (38.75cm X 29.21cm) with cap size 70mm (CP Lab Safety, UK) which was sectioned in a longitudinal plane (i.e. to form a drum as shown approximately ¾ depth of the original). An anaesthetic machine 'Minimon 7138B', (Kontron Medical) was used to measure the intraluminal pressure via a DTX Plus™ pressure transducer (BD, USA). A water pump was used to pump the fluid into the intestine.

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